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THE INFLUENCE OF CHILLING, ABOVE THE FREEZING POINT, ON CERTAIN CROP PLANTS[1]

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INTRODUCTION

It has been held that short periods of cold weather above the freezing point cause injury to crop plants of tropical or subtropical origin. It is commonly known that growth is retarded by such temperatures but it does not seem to be generally conceded that definite lesions may result. The authors, therefore, thought it worth while to investigate the effect of temperature near, but above, the freezing point on certain crop plants and, if injury were found to occur, to study the relative susceptibility of different crops and varieties and the circumstances under which injury takes place.

REVIEW OF LITERATURE

Very little recent literature has been contributed to this subject. Molisch (*12*),[2] 1896, critically reviewed the earlier literature, making special reference to the work of Bierkander (*2*), Goeppert (*7*), Hardy (*8*), Sachs (*19*), and Kunisch (*10*), and conducted rather extensive experiments of his own. Some 58 plants from subtropical regions were examined and found to be injured by temperatures ranging from 1° to 7° C. Some showed characteristic black spots on their leaves; in others the leaves rolled and ultimately dropped off, some plants died and some survived without injury.

Sachs attributed the injury to the inability of the roots to absorb and convey sufficient water from the cold soil to the leaves to make up for the transpiration deficit and hence the plants died of drought. But Molisch disproved this for *Episia*, *Sanchezia*, and some 10 other plants, by showing that even when they were kept in air having an average humidity of 98 per cent, thus reducing transpiration to a minimum, the characteristic injury nevertheless occurred.

Faris (*5*), working on sugar cane in Cuba, reported the appearance of "mancha blancas" or white bands 2 to 4 inches wide across the leaves in which the tissues seemed to have lost the power to form chlorophyll. A study of the weather conditions showed that the bands occurred when low temperatures followed periods of rainfall. He thought that rain stimulated growth and the cold water standing in the curl of the leaves chilled the newly exposed tender tissues and thus caused chlorosis. Cold weather when not preceded by rainfall caused only slight chlorotic bands. Marked varietal differences were found and some 20 varieties were arranged on a scale of 1 to 10, according to their resistance. In Habana Province, where this study was made,

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[2] Reference is made by number (italic) to "Literature cited," p. 337.

no freezing temperatures have ever been recorded. He concludes that a temperature from 5° to 10° C. for two to three nights is sufficient to cause white bands on susceptible plants. According to Faris, cold chlorosis has also been reported from Australia, Hawaii, and Louisiana.

Faris was able to produce similar bands artificially by tying a paper funnel around the plant shoot and filling it with ice for three consecutive nights. The white bands developed six days later.

Collins (*4*) found that albinism in certain varieties of barley could be induced by growing the plants at temperatures below 45° F. (7.3° C.). When the plants were grown at temperatures above 65° chlorophyll developed normally.

Collings (*3*) states that cool nights, but without frost, injure, dwarf, and delay growth of young cotton plants.

Marcarelli (*11*) observed that the yellowing of the young plants of late rice, irrigated with cold water in the upper Vercelli district, Piedmont, Italy, was caused by frequent early morning low temperatures accompanied by mist.

Pantanelli (*15*) studied the relation between various salts and the injury to plants exposed to temperatures near the freezing point. Wheat, beets, and sunflowers were exposed to freezing temperatures and maize and tomatoes to temperatures "a little above 0° C." Different lots in each case were supplied with sodium, potassium, ammonium, and magnesium salts. The same relations were observed whether death resulted from freezing or from cooling alone. The author concludes that "resistance to cold has, therefore, no connection with the concentration of the cell sap, nor with its content in acids or salts, but with the amount of sugar retained by the cell during cooling."

EXPERIMENTAL RESULTS

The investigations reported herein were carried out during the summer of 1927 in the greenhouses of the agricultural experiment station, Manhattan, Kans. Good ventilation was afforded and excessive temperatures were prevented by covering the glass with a coating of whitewash (calcium hydroxide) which, however, excluded very little light. The plants not subjected to chilling or other abnormal conditions made normal growth and seed produced. Even soy beans, which often grow thin stemmed and decumbent in greenhouses, were in no way different from field-grown plants.

The plants were grown in wooden flats or boxes 24 by 24 by 4 inches and in about 2,000 red clay 4-inch greenhouse pots. They stood for most of the time on the greenhouse floor. Their position was changed every third day to equalize conditions dependent upon location. Thick plantings were made and later thinned to one sturdy plant to each pot or each 12 square inches in the flats.

Soy beans and velvet beans were germinated in coarse quartz sand and the more vigorous seedlings transplanted to pots on account of the scarcity and low vitality of the seed. Twenty-five to one hundred pots were planted at intervals throughout the time these experiments were in progress, with the object of having plants 2 to 3 weeks old whenever needed.

The chilling was effected in a chamber specially constructed in the greenhouse for low-temperature investigations. (Fig. 1.) Its walls are constructed of sheet cork, protected on the outside by plaster and cement, the entire thickness of cork and plaster being 12 inches. The

chamber was provided with three doors, each 42 by 24 inches, which fitted snugly into the openings they covered. These doors were closed during the night only. During the day they were replaced by double glass fitted into wooden frames, the glasses being separated by a space of 3 inches. The chamber was approximately 10 feet long, 4 feet wide, and 3 feet deep. The coils cooling the chamber were 6 inches away from the four walls and the floor, leaving a space of approximately 67 cubic feet between them.

Wooden boards were placed lengthwise on cross pieces 6 inches from the floor. On these boards the pots were placed in juxtaposition and at least a pot diameter away from the coils. In no instance were the plants allowed to touch the coils. Plants from each set similarly treated were placed across the chamber so that each group had an equal number of plants in the middle and toward the sides. This precaution

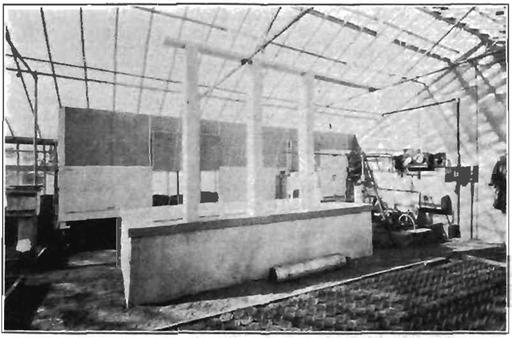


Fig. 1.—Cold chamber and refrigeration plant used in chilling crop plants

proved unnecessary since comparisons of three thermometers over a period of four days indicated that there was no appreciable difference in temperature for any length of time in different sections of the chamber.

Cooling was effected by a carbon dioxide refrigeration plant thermostatically controlled. By this arrangement it was possible to maintain the desired temperatures within a range of about three centigrade degrees. The temperatures within the chamber and in the greenhouse were continuously recorded by two Tycos thermographs, which were checked twice daily with minimum and maximum thermometers suspended near them.

Watering of the plants before and after chilling was done daily but at no regular time, approximately 40 to 50 c. c. of water being applied to each plant. In all instances (except where the quantity of water used was the subject of investigation) water was applied immediately before the plants were placed in the chilling chamber. While the plants were being chilled they received no water. Those pots which were saturated prior to chilling were watered a sufficient time before chilling to allow all the free water to drain off.

The humidity of the air within the chamber ranged, as measured with a Tycos hygrodeik, between 95 and 98 per cent. Sufficient moisture condensed on the refrigeration pipes to warrant a triweekly scooping out of the water from the chamber floor. While the pots were in the chamber they remained so moist that watering was unnecessary even over a five-day period. It seems likely that very little if any transpiration took place.

The opening of the doors, replacing and lifting of the glass frames, together with fluctuating temperatures in the chambers, no doubt forced in and withdrew sufficient air to effect a regular exchange between the inside and the outside. Under such circumstances the air surrounding the plants was not very different from that in the greenhouse.

METHOD OF RATING INJURIES

The amount of injury, retardation of growth, and other abnormalities induced by chilling were recorded, in most instances, within 24 hours after the plants were removed from the chamber while the immediate effects were most clearly visible, and again after two or three weeks. All records of injury were made on a percentage scale. Each species reacted in its own particular way, and this fact had to be considered in estimating the amount of injury that had taken place. The first notes were based on the proportion of leaf and stem surface showing visible injury. Final notes were based on the estimated permanent injury to the plant. Thus a plant having a few dry tips or drooping petioles, depending on the total number of leaves of the plant, was rated as 5 to 10 per cent injury; whereas a total loss of all leaves might be rated at 95 per cent and of the stem and all leaves at 100 per cent. After a little practice there appeared to be no difficulty in assigning approximately the same figure to plants which had been similarly injured at different times. As a rule plants recorded soon after chilling as having suffered 25 per cent injury or less recovered and were capable of seed production. Those injured more than 25 per cent and less than 50 recovered occasionally. Plants recorded as 50 per cent or more injured seldom recovered.

SCOPE OF THE INVESTIGATION

Preliminary tests were first conducted to find what plants might be expected to suffer injury and what temperature and periods of exposure were necessary to induce such injury. More careful experiments were then undertaken to determine not only the temperature and duration necessary to produce injury, but also the relative susceptibility of different crops and varieties as well as the effect of various salt solutions on the degree of injury. The effect of varying amounts of water in the soil was also investigated. Summer crop plants, mostly of subtropical origin sensitive to the least degree of frost, were included in this study. Unless otherwise indicated they were grown from Kansas-produced seed. The following plants were used:

Cowpeas. *Vigna catjang sinensis:* Early Buff and Whippoorwill varieties from Georgia.

Velvet beans. *Stizolobium deeringianum:* Ordinary mottled variety from Georgia.

Peanuts. *Arachis hypogaea:* Valencia and Virginia Bunch varieties; source of seed unknown. Spanish from Georgia.

Soy beans. *Soya max:* Manchu and Virginia varieties.
Cotton. *Gossypium hirsutum:* Westex from Texas. Trice from North Carolina. Delfos from Mississippi. Oklahoma Triumph from Oklahoma.
Sunflower. *Helianthus annus:* White-seeded variety.
Tomato. *Lycospersicum esculentum validum.*
Potato. *Solanum tuberosum.*
Flax. *Linum usitatissimum.*
Watermelons. *Citrullus* sp.
Pumpkin. *Curcubita pepo.*
Tepary bean. *Phaseolus acutifolius* (A. Gray) *latifolius* (Freeman).
Buckwheat. *Fagopyrum esculentum.*
Maize. *Zea mays:* Midland Dent, Colby and F_1.
Kansas sunflower Hybrid No. 5413–1 × 5412–1.
Sorghums. *Holcus sorghum.* Blackhull kafir, Kansas Orange.
Rice. *Oryza sativa:* Early Prolific and Honduras varieties from California.
Sudan grass. *Andropogon sudanense.*
Teff grass. *Eragrostis abyssinica:* From the Union of South Africa.

EXPERIMENTAL RESULTS

The outstanding result of the investigation was the very evident injurious effects of chilling [13] on certain plants and the high degree of resistance shown by others. A point of considerable interest was the reaction of different species and varieties not only to difference in the degree of temperature, but also with respect to the nature of the injury. It seems desirable, therefore, to describe briefly the nature of the injury in each case.

NATURE OF THE INJURIES

The most obvious effect of chilling on susceptible plants was the drying and falling off of the leaves or portions of leaves in a way very similar to that which follows frost damage. The effect, however, in all except velvet beans, took place much more slowly, being apparent only from one to several days after chilling. In velvet beans, the effect was visible an hour or two after chilling when the leaves of young plants showed light purplish or purplish to black discoloration. This condition was followed by the drying of the leaves in the course of two to three days. When the exposure was brief there was no immediate effect, but in the course of five to seven days light brown areas appeared on the leaves. Plants 4 to 5 weeks old showed no abnormalities at first, but in a day or two a very pronounced chlorotic condition set in throughout the entire plant, from the top downward, accompanied by the dropping of the turgid leaves.

In most cases the effect on cowpeas was not immediately visible, except that when they came out of the chilling chamber the leaves were partly folded. A chlorotic condition, confined to the older leaves and more especially to leaf sections and similar to that in the velvet bean plants, usually developed within 36 hours. The leaves dropped while turgid and for the greater part still green. Slight injuries were evident in the form of disrupted or blistered areas confined to the edges of the younger leaves. (Pl. 1.) These areas eventually dried, turned brown, and disintegrated, leaving a leaf with a jagged outline. Cowpea plants were seldom killed outright, although the leaves were easily injured and the growth of the roots was considerably reduced. (Fig. 2.) New growth was generally produced from uninjured stem buds.

[13] Chilling in this study refers in all cases to temperatures above zero centigrade. In no case was the temperature allowed to go below zero.

The after effects of chilling manifested by cotton and soy beans were very similar, although as pointed out later, there was the most marked difference in the degree of injury. The most striking effect was the presence of spots or fringes of light green which later became white and coriaceous. To the casual observer such leaves would seem to have been injured by frost except that the entire leaf was seldom affected. In severely injured leaves and occasionally in others a narrow brick-red band separated the living and dead portions of the leaves. (Pl. 1.)

Peanuts showed no obvious effects immediately after chilling. After about three days the tops wilted more and more during the afternoons, recovering somewhat at night. Wilting and recovery alternated until the plants died or recovered completely. Later examination showed that the root system had been seriously injured.

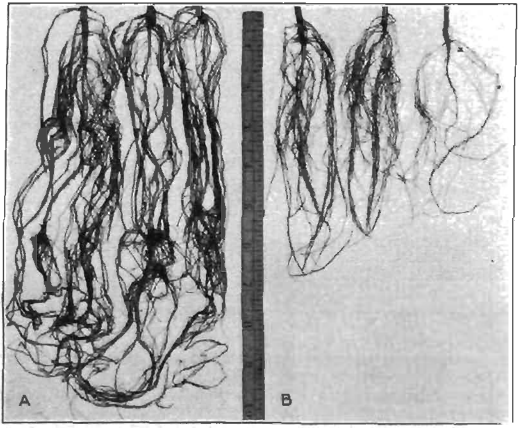
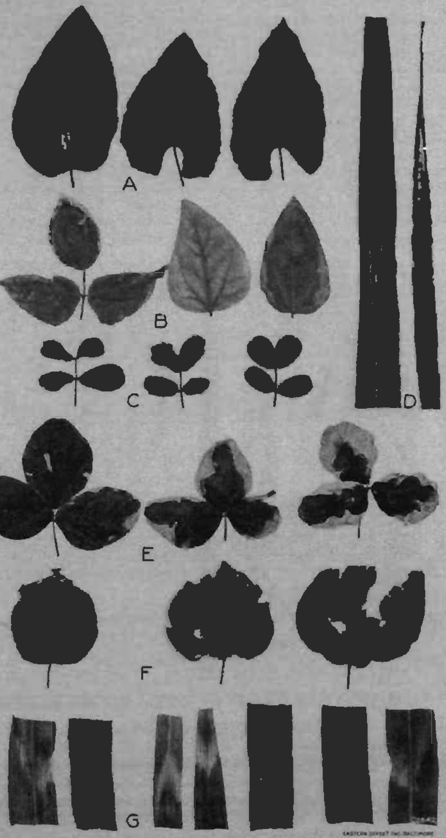


FIG. 2.—The effect of chilling on the roots of 3-week-old Whipporwill cowpea plants. A, not chilled; B, chilled for 36 hours at 2° to 4° C. Plants 6 weeks old when photographed

(Fig. 3.) In the case of young Virginia Bunch peanut plants a few leaflets developed narrow, dry brittle borders. More often individual leaflets became much dwarfed in comparison with the normal ones next to them. (Pl. 1, bottom.) In general the tops were uninjured. Injuries to the root systems, however, as will be shown later, were sufficient greatly to retard growth and in some cases to cause the death of the plants.

In watermelons and pumpkins the margins of the leaves turned light green and ultimately brown. When severely chilled the plants were unable to remain upright, the leaves then turning slightly yellow and folding inward. Chilling very clearly decreased the rate of growth but otherwise there was no marked effect either on the roots or leaves.



TYPES OF INJURY SUSTAINED BY VARIOUS PLANTS WHEN CHILLED

A, velvet bean basal leaves, a leaf from a slightly injured plant and two leaves from severely injured plants; B, Early Buff cowpeas, terminal leaf from a slightly injured plant and two leaves from severely injured plants; C, peanut leaves, showing characteristic dwarfing of leaflets and fringing that occurs after chilling; D, irregular Faris band on Kansas Orange sorghum leaf and fringed tip of the same; E, leaves from Virginia soy-bean plants which were chilled 48, 72, and 96 hours, respectively; F, Delfos cotton leaves which were chilled 48, 72, and 96 hours, respectively; G, Faris or chill bands on maize leaves from plants that have been chilled 60 hours, at 2° to 4° C.

Buckwheat and Tepary beans were severely wilted during and immediately after chilling, appearing as though they had been frosted. They recovered later to the extent that after a week or two scarcely any injury was perceptible.

Potatoes, tomatoes, and flax showed no appreciable injury or retardation in growth in any part during or after chilling, even when chilled as long as 120 hours. In a few tomato plants some half-developed leaf tips lost their turgidity and became pale green. Recovery in every case was very rapid.

Chilling had no marked immediate effect on maize, sorghum, Sudan grass, or Teff grass. From 5 to 10 days after chilling there developed on the leaf blades light yellow transverse bands, varying in size and intensity of color, similar to those described by Faris (*5*) in sugar cane. Their breadth and number per plant were proportional to the duration of the chilling. With advancing age these areas became transparent, filmy, and rust colored toward the edges. (Pl. 1, bottom.) It was evident that these bands occurred on those parts of the blades which formed the curl of the plant at the time of chilling. In this region the most active growth takes place and, incidentally, here the youngest and most easily injured tissue is to be found. Plants severely injured allowed their leaves to roll or droop as a browning of the entire plant set in and when dead appeared not unlike plants that had succumbed to drought or to intense heat.

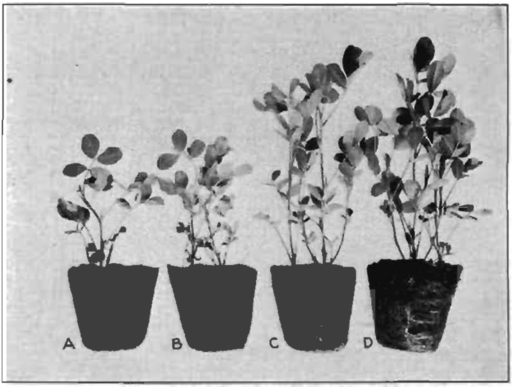


FIG. 3.—Retarded growth of Spanish peanuts due to root injury caused by chilling. A and B, chilled for 84 hours; C and D, not chilled

Microscopic examination of maize root sections from chilled and unchilled plants showed brown lesions on the roots of chilled plants only. Mycelia were found in apparently healthy root sections of chilled plants, while plants not chilled failed to reveal any abnormalities either internally or externally.

Unlike the foregoing, rice plants became yellow first in the leaf sheaths and then in the upper halves of the blades. When the chlorotic condition was confined to the sheaths, the plants recovered but when more extensive they perished. They did not develop Faris bands, nor did they show injuries on the roots. The yellowed sheaths invariably peeled away from the leaves they enveloped, becoming somewhat white, while their blades developed streaks of yellow and faint red. Cessation of normal functioning and discoloration took place alike throughout the entire plant, without any one part showing especially characteristic markings of its own.

RELATION OF PERIOD OF EXPOSURE TO INJURY

One of the first points to be investigated was the duration of exposure necessary to produce damage. For this study various plants were exposed to temperatures slightly above freezing for various periods of time ranging in general from 12 to 132 hours. Six to ten plants were used as a unit and the temperature in the chilling chamber was 0.5° to 5° C. in most cases. Exceptions are noted elsewhere. Tables 1 to 3 give averages of the estimated percentage injury sustained by the plants, as well as the least and greatest percentage injury in each set.

TABLE 1.—*The effect of chilling at 0.5° to 5.° C. on legumes*

Length of time chilled at 0.5° to 5° C. (hours)	Injury to plants: 24 hours after chilling		Injury to plants: 2 weeks after chilling		Height of plants 4 weeks after chilling	
	Average per plant	Range	Average per plant	Range	Average	Range
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Inches*	*Inches*
VELVET BEANS (3 WEEKS OLD)						
12	0	0-00	4.4	0-10		
24	11.4	8-30	46.4	30-100		
36	42.4	10-50	80.0	60-100		
48	60.3	10-90	98.0	100-100		
EARLY BUFF COWPEAS (3 WEEKS OLD)						
12	0	0-0	3.0	3-3		11-13
24	3.0	0-9	10.0	10-10		13-14
36	26.5	15-30	20.0	20-20		12-14
60	45.0	44-55	40.0	40-40		11-12
84	55.7	40-60	60.0	60-60		9-10
96	77.0	70-90	96.0	96-96		5-7
SPANISH PEANUTS (3 WEEKS OLD)						
0					6.1	5-7
36	0.7	0-3	5.3	0-8	5.2	3.5-6
48	5.0	4-9	70.0	35-90	3.6	3-4.5
84	10.0	5-12	70.5	15-90	3.3	3-4
96	4.0	4.5	88.5	70-100	3.5	2.5-4
120	7.8	6-12	95.8	90-100	2.8	2-4
MANCHU SOY BEANS (4 WEEKS OLD)						
84	0	0-0	0	0-0		
120	0	0-0	0	0-0		
VIRGINIA SOY BEANS (3 WEEKS OLD)						
48	6.2	0-10	4.8	0-10	20.1	12-26.5
72	44.0	25-60	40.0	20-100	17.7	10-23
96	45.0	15-70	39.0	20-80	16.1	10-23
132	51.3	0-70	60.0	20-100	14.4	9-17
TEPARY BEANS (2 WEEKS OLD)						
48	5.0	5-5	0	0-0		
84	80.0	80-80	5.0	5-5		

TABLE 2.—*The effect of chilling at 0.5° to 5° C. on sorghums and maize*

Length of time chilled at 0.5° to 5° C. (hours)	Injury to plants: 24 hours after chilling, Average per plant	Injury to plants: 24 hours after chilling, Range	Injury to plants: 2 weeks after chilling, Average per plant	Injury to plants: 2 weeks after chilling, Range	Height of plants 4 weeks after chilling, Average per plant	Height of plants 4 weeks after chilling, Range
BLACKHULL KAFIR (5 WEEKS OLD)						
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Inches*	*Inches*
0	----------	0–0	----------	----------	19.6	18 –20
24 [a]	0	0–0	3.0	0–5	19.1	18 –19
48	0	0–0	4.0	3–5	16.5	15 –18
72	0	0–0	4.3	2–6	17.2	15.8–18
BLACKHULL KAFIR (6 WEEKS OLD)						
24 [a]	0	0–0	3.0	5–10	----------	----------
48 [a]	0	0–0	5.4	5–7	----------	----------
60	0	0–0	3.0	0–15	----------	----------
72	0	0–0	10.0	10–10	----------	----------
KANSAS ORANGE SORGO (6 WEEKS OLD)						
0	0	0–0	0	0–0	18.0	16 –21
24 [a]	0	0–0	0	0–0	21.6	16 –22
48 [a]	0	0–0	2.2	3–5	19.1	16.5–22
60	0	0–0	5.9	0–10	18.8	16 –22
72	0	0–0	5.1	0–12	16.2	15 –18
MIDLAND YELLOW DENT MAIZE (6 WEEKS OLD)						
0 [a]	0	0–0	0	0–0	38.3	31 –40
24	5.0	5–5	5.0	5–5	0	26 –36
48 [a]	10.0	10–10	15.0	15–15	0	22 –33
60 [a]	20.0	20–20	20.0	20–20	0	22 –31
72 [a]	30.0	30–30	25.0	25–25	28.8	21 –37

[a] 5 plants only.

These data make it clearly evident that some plants are very sensitive to chilling, while others are exceedingly resistant. Thus an exposure of 12 hours produced noticeable injury in rice and velvet beans, and 24 hours or longer caused severe injury. (Fig. 4, top.) Early Buff cowpeas were severely injured by an exposure of 60 hours or longer. (Fig. 5, top.) Peanuts, Sudan grass, and Teff grass were slightly injured by a 24-hour exposure, severe injury occurring only when chilled for more than 36 hours. Peanuts reacted differently from the others in this group in that the effects were not apparent until several days after the plants were chilled, due to the fact that the roots and not the tops were injured. (Fig. 5, bottom.) Maize, sorghums, watermelons, and pumpkins were hardier, for after 48 hours exposure they showed only about 10 to 15 per cent injury. The remainder of the plants included in this investigation can be considered as highly resistant to chilling. Thus, buckwheat, Tepary beans and Virginia soy beans were injured only 10 per cent when chilled for 60 hours, and potatoes and sunflowers exhibited no injury when chilled for twice that period. The ability of many of these crops, especially tomatoes, to resist chilling is of special interest in view of their frequent inability to survive subzero temperatures.

TABLE 3.—*The effect of chilling on miscellaneous plants*

Number of plants	Age in weeks	Kind of plant	Time chilled	Temperature	Percentage injury 48 hours after chilling	Height of plants 3 weeks after chilling
			Hours			*Inches*
20	2	Early Prolific rice	12	2° to 4° C.	20	8–12
20	2	do	24	do	42	6
20	2	do	36	do	96	Dead.
20	2	do	48	do	100	Dead.
20	2	do	72	do	100	Dead.
20	2	Honduras rice	12	do	12	8–12
20	2	do	24	do	18	6
20	2	do	36	do	25	Dead.
20	2	do	48	do	100	Dead.
20	2	do	72	do	100	Dead.
20	3	Sudan grass	12	do	10	11–16
20	3	do	24	do	10	11–15
20	3	do	36	do	20	11–15
20	3	do	48	do	35	9–11
20	3	do	72	do	50	7–9
20	3	Teff grass	12	do	11	12–16
20	3	do	24	do	20	12–16
20	3	do	36	do	15	11–13
20	3	do	48	do	35	9–10
20	3	do	72	do	50	Dead.
6	4	Watermelon	36	0.5° to 5° C.	10	
		do	60	do	60	
6	4	Pumpkin	48	do	15	
		do	84	do	55	
12	4	Buckwheat	84	do	5	
6	3	Tomato	36	do	5	
		do	84	do	5	
6	8	do	36	do	5	
		do	120	do	0	
12	4	Potato	84	do	0	
6	4	Sunflower	84	do	0	
		do	120	do	0	
30	3	Flax	84	2° to 4° C.	0	
		do	120	do	0	

RELATION OF TEMPERATURE TO INJURY

Several of the plants which were found to be exceptionally sensitive, namely Delfos cotton, velvet beans, and Whippoorwill cowpeas, were subjected to a temperature range of 5° to 10° C. for various lengths of time. The injury sustained by each at the various intervals is given in Table 4. It is evident that severe injury occurred at the higher temperatures, but as would be expected a longer period of exposure was necessary. Whippoorwill cowpeas were especially sensitive at this temperature, being rather severely injured by exposure for 48 hours, whereas Delfos cotton and velvet beans survived exposures of the same period without severe injury. This is of special interest in view of the fact that velvet beans were very sensitive at the lower temperatures. As at the lower temperatures, more damage was apparent two weeks after chilling than 24 hours after. Altogether, the results may be taken to show that injurious effects may be brought on with rather brief exposures at temperatures considerably above the freezing point.

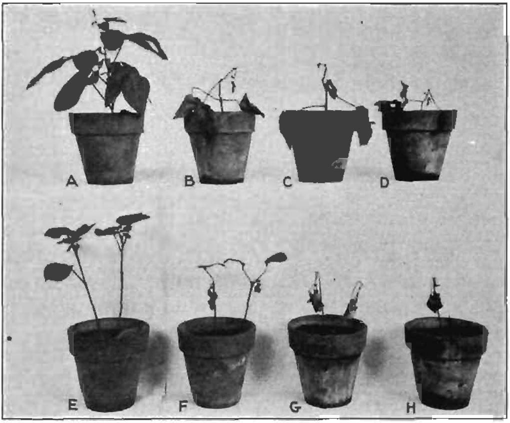


FIG. 4.—Injury to velvet beans (top row) and Delfos cotton (bottom row) when plants were chilled at 0.5° to 5° C. A–E, B–F, C–G, and D–H were chilled 12, 36, 60, and 84 hours, respectively

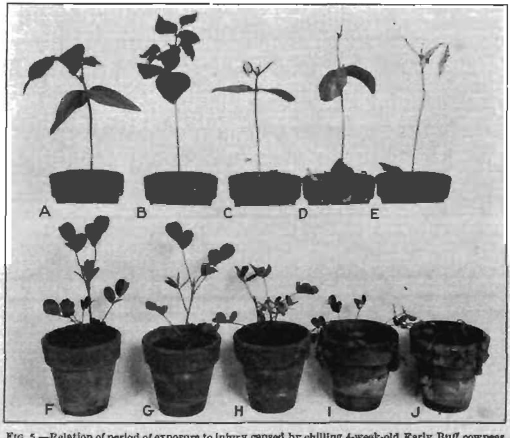


FIG. 5.—Relation of period of exposure to injury caused by chilling 4-week-old Early Buff cowpeas (top row) and 4-week-old Spanish peanuts (bottom row) at 0.5° to 5° C. A–F, B–G, C–H, D–I, and E–J were chilled 0, 36, 48, 84, and 96 hours, respectively

TABLE 4.—*The effect of chilling at 5° to 10° C. on cotton, velvet beans, and cowpea*

Length of time chilled at 5° to 10° C. (hours)	Injury to plants: 24 hours after chilling, Average per plant	Injury to plants: 24 hours after chilling, Range	Injury to plants: 2 weeks after chilling, Average per plant	Injury to plants: 2 weeks after chilling, Range
	Per cent	*Per cent*	*Per cent*	*Per cent*
DELFOS COTTON				
48	0	0–0	25.0	25–25
72	3.0	0–12	30.0	30–30
96	7.7	0–12	38.0	35–40
120	51.0	25–60	78.0	40–100
VELVET BEANS				
48	1.8	0–8	1.6	0–6
72	12.2	0–30	9.0	0–30
96	40.0	35–50	42.5	10–100
120	73.0	35–90	86.0	50–100
WHIPPOORWILL COWPEAS				
24	0	0–0	40.0	40–40
48	41.6	20–50	41.6	20–50
72	59.0	35–70	70.0	60–100
96	71.8	65–80	100.0	100
120	92.1	90–96	100.0	100

VARIETAL DIFFERENCES

That there may be important varietal differences has been suggested by the data already presented. In view of the importance of this subject in relation to varietal adaptation and improvement, some tests were made to determine the degree of such differences. Three varieties each of cotton and peanuts were therefore chilled for various periods of time with the results presented in Table 5.

TABLE 5.—*The effect of chilling on varieties of cotton and peanuts*

Variety and age of plants; temperature and duration of chilling	Percentage injury: 24 hours after chilling	Percentage injury: 2 weeks after chilling	Percentage injury: 3 weeks after chilling
Cotton plants (3 weeks old):			
Chilled 24 hours at 2° to 5° C.—			
Trice	60	45	30
Delfos	75	56	40
Westex	65	60	45
Chilled 84 hours at 2° to 8° C.—			
Trice	55	70	70
Delfos	80	85	80
Westex	90	90	95
Chilled 96 hours at 5° to 10° C.—			
Trice	40	70	55
Delfos	75	85	80
Westex	85	95	100
Chilled 108 hours at 5° to 10° C.—			
Trice	60	75	30
Delfos	75	75	70
Westex	85	85	85
Average of 4 exposures—			
Trice	54	65	46
Delfos	76	75	67
Westex	81	82	81
Peanut plants (5 weeks old):			
Chilled 84 hours at 0.5° to 5° C.—			
Virginia Bunch	15	25	12
Spanish	54	88	100
Valencia [a]	68	95	100
Peanut plants (8 weeks old):			
Chilled 144 hours at 2° to 10° C.—			
Virginia Bunch [a]	43	90	
Spanish [a]	60	95	
Valencia [a]	71	100	

[a] 5 plants only.

It is very evident that varieties of cotton and peanuts differ greatly in their ability to resist the influences of chilling. Trice cotton proved to be most hardy followed by Delfos, while Westex was the most sensitive. Thus an average for all trials was 46 per cent injury for Trice, 67 per cent for Delfos, and 81 per cent for Westex. (Fig. 6, bottom.)

But even greater differences were exhibited between varieties of peanuts. Virginia Bunch was exceptionally hardy, while Valencia and Spanish were very sensitive. Thus an exposure of 84 hours was fatal to Valencia but scarcely injured the Virginia Bunch. Spanish was intermediate. The same relation obtained with an exposure of 144 hours. (Fig. 6, top.)

Attention has already been called (Tables 1 to 4) to the susceptibility of Whippoorwill cowpeas, a variety from Georgia which was

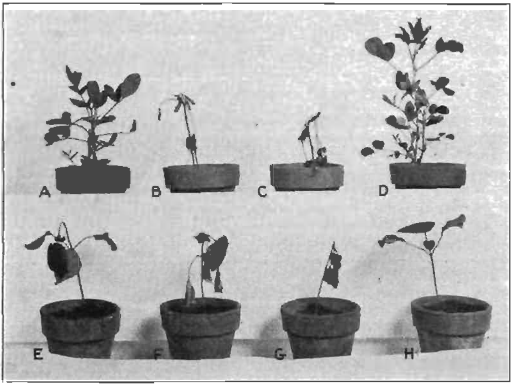


FIG. 6.—The effect of chilling on typical plants of varieties of peanuts (top) and cotton (bottom). Left, to right, top row, peanut plants chilled at 05° to 5° C. for 120 hours: A, Virginia Bunch; B, Spanish; C, Valencia; and D, Spanish peanuts not chilled. Botton row, cotton plants chilled at 2° to 8° C. for 84 hours: E, Trice; F, Delfos; G, Westex, and H, Delfos cotton not chilled

injured far more than Early Buff from Kansas, although exposed to a temperature approximately 5° higher.

Important differences were also observed in rice, Honduras being injured decidedly less than Early Prolific. (Table 3.) Similar differences were observed with soy beans. Virginia soy beans were severely injured by an exposure of 72 hours, whereas Manchu survived an exposure of 120 hours without injury.

RELATIVE INJURY TO DIFFERENT CROPS

The plants included in this investigation can be conveniently arranged into five classes according to their manner of reacting to low temperatures. This classification is based on the degree of injury as well as on the particular mode of reaction exhibited by each species. Such a classification must of course be considered as dis-

tinctly preliminary, owing to the incompleteness of the investigation, and is presented only for convenience.

In class 1 are included those plants which are killed by an exposure of 60 hours to temperatures from 0.5° to 5° C. They are rice, velvet beans, cowpeas, and cotton.

In class 2 are included those which are decidedly injured by such temperatures but which with favorable conditions will recover. This class includes Sudan grass, Teff grass, Spanish and Valencia peanuts.

Class 3 includes those which in general are not likely to suffer serious injury by the conditions specified above. They are Virginia Bunch peanuts, maize, sorghum, watermelons, and pumpkins.

Class 4 includes those which are noticeably injured by prolonged chilling, but in which injury is likely to be nominal, namely, buckwheat, Tepary beans, and soy beans.

Class 5 includes those plants which when exposed at 0.5° to 5° C. were not injured so far as could be observed. They are potatoes, sunflowers, tomatoes, and flax.

THE RELATION OF MOISTURE TO CHILLING INJURY

In order to learn what effect the amount of moisture in the soil had on the percentage of injury, various amounts of water were added for five days before the plants were chilled. Water was withheld 24 hours prior to the application of any treatment. The normal amount of water the plants received each day was 35 to 40 c. c. This was sufficient to keep the soil in each 4-inch pot more or less saturated. The least amount applied was 10 c. c. per pot per day, this being the minimum with which plants could be kept alive. In all, the following amounts were applied in various experiments, namely, 10, 15, 20, 23, 40, 55, 90, 120 c. c. daily and in addition 90 c. c. daily with six hours submersion immediately before chilling. The results as given in table 6 show that the amount of moisture in the soil had a marked effect on chilling injury.

In almost every instance plants were far more severely injured in saturated than in moderately wet soil. In very dry soil they suffered slightly more than in moderately dry, but considerably less than in saturated soil. Thus the injury two weeks after chilling to Velvet beans exposed for 18 hours was 14 per cent in dry soil and 80 per cent in wet soil. Corresponding figures for Early Buff cowpeas exposed for 36 hours were 20 per cent and 40 per cent, and for Spanish peanuts exposed for 84 hours 20 per cent and 75 per cent, respectively. Manchu soy beans were injured slightly more in dry soil, but this may possibly have been due to drought. Cowpea plants were very slow to recover when they were chilled in dry soil, even though they showed here the least reaction; when chilled in saturated soil they recovered very quickly considering the degree of injury.

These observations seem to be in line with the common practice of hardening garden plants by drying prior to transplanting.

The influence of various amounts of moisture in determining the nature of the reaction suggests that the contention often advanced that plants are more severely injured by low temperature during wet weather is by no means amiss; also, that when low temperatures occur in dry weather the effect of chilling is likely to be less severe than if the weather were wet.

TABLE 6.—*The relation of soil moisture to the effect of chilling on various plants*

Plant variety	Chilling temperature	Amount of water applied to plants	Injury to plants: 24 hours after chilling: Average per plant	Injury to plants: 24 hours after chilling: Range	Injury to plants: 2 weeks after chilling: Average per plant	Injury to plants: 2 weeks after chilling: Range	Height of plants: Average per plant	Height of plants: Range
	° C.	*C. c.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Inches*	*Inches*
Velvet beans (3 weeks old).	84 hours at 0.5° to 5°.	15	44	20-60	Died.	Died.	....	....
		23	58	20-75	Died.	Died.	....	....
		55	68	25-95	Died.	Died.	....	....
		90	85	70-94	Died.	Died.	....	....
Velvet beans	18 hours at 2° to 4° C.	20	75	0-20	14	5-20	....	....
		40	32	10-85	38	15-100	....	....
		120	83	65-100	80	60-100	....	....
Manchu soy-beans (4 weeks old).	84 hours at 0.5° to 5° C.	15	12.4	10-20	13.0	5-20	15.7	11-19
		23	7.0	7-7	2.6	2-3	20.0	17-24
		55	2.0	1-4	1.4	0.3	25.4	22-28
		90	1.2	1-2	4.6	4-6	27.4	22-31
		[a] 90	0	0-0	.0	0-0	24.0	17-22
Black Eye cowpeas (4 weeks old).	84 hours at 0.5° to 6° C.	15	46	40-50	49	45-50	6.8	6-7
		23	50	40-60	80	80-80	6.6	5-8
		55	60	25-80	86	75-90	9.2	10-11
		90	80	75-90	80	80-80	10.4	10-11
		[a] 90	79	50-80	80	80-85	8.8	7-9
Early Buff cowpeas (3 weeks old).	36 hours at 2° to 4° C.	10	30	30-30	20	....	8.2	6-9
		20	40	40-40	30	....	8.5	7-10
		40	40	40-40	40	....	8.5	7.5-10
Delfos cotton (3 weeks old).	18 hours at 2° to 5° C.	10	2.2	0-5	5	5-5	6.1	6-7
		20	3.0	0-10	10	10-10	5.6	5.5-6
		40	11.8	0-25	20	20-20	6.3	5.5-7
Spanish peanuts (3 weeks old).	60 hours at 2° to 4° C.	10	0	0	7	5-12	3.5	2-5
		20	0	0	12	0-20	4.4	2-5
		40	0	0	25	25-25	4.7	3-5
Spanish peanuts	84 hours at 2° to 4° C.	10	0	0	20	20-20	....	....
		20	0	0	60	60-60	....	....
		40	0	0	75	75-75	....	....
Maize (Kansas sunflower F_1 hybrid (3 weeks old).	36 hours at 2° to 5° C.	10	0	0	6	5-10	9.5	9-10
		20	0	0	35	35-35	10.3	9-12
		40	0	0	50	50-50	9.5	9-10
Maize (Kansas sunflower F_1 hybrid.	60 hours at 2° to 5° C.	10	0	0	7	5-15	....	....
		20	0	0	42	40-45	....	....
		40	0	0	50	50-50	....	....

[a] Submerged for 6 hours prior to chilling.

INFLUENCE OF CHILLING ON PLANTS OF DIFFERENT AGES

In the greater part of this investigation plants were chilled when 3 weeks old. In order to determine the relative sensitiveness of plants at different ages, velvet beans, Delfos cotton, and Black Eye cowpeas of various ages were chilled with the results as given in Table 7.

This experiment shows that in general the younger the plants, the more they were injured. Velvet beans, 28 days old, chilled for 24 hours at 0.5° to 5° C. were injured only 9.4 per cent, while plants half this age showed double the amount of injury and 9-day-old plants were killed. Similar though much less marked differences were observed with the Black Eye cowpeas. As for the cotton plants there was a tendency for them to be most hardy when 35 days old. Even as short a chill as 24 hours at 2° to 4° C. proved fatal to many 10-day-old plants, whereas 17- and 25-day-old ones succumbed only after 48 hours. (Fig. 7.) However, plants 58 days old withered, dropped their leaves, and were injured in all cases more than those 35 days old.

That young plants and the newer parts of old plants suffered more than the full-grown plants and mature parts seems not unnatural. It would follow from this that the changes which ultimately have a detrimental influence occur where the most vigorous growth is taking place, and where cell activity is most intense.

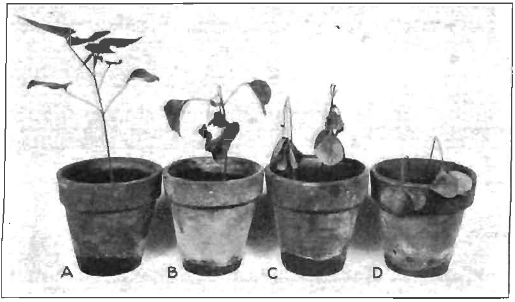


FIG. 7.—Injury to Delfos cotton plants of various ages caused by chilling at 1.5° to 5° C. for 96 hours Left to right: A, 26 days old, not chilled; B, C, and D, 26, 19, and 10 days old, respectively, photographed 24 hours after chilling

TABLE 7.—*The effect of chilling on plants of different ages*

Plant variety	Chilling temperature	Age of plants	Injury to plants: 24 hours after chilling: Average per plant	Injury to plants: 24 hours after chilling: Range	Injury to plants: 2 weeks after chilling: Average per plant	Injury to plants: 2 weeks after chilling: Range
	°C.	*Days*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Velvet beans	24 hours at 0.5° to 5°	9	51	30-85	----------	100
		14	20	15-30	----------	80-100
		28	9.4	5-25	----------	70-100
Do	36 hours	9	61	50-85	----------	100
		14	25	20-30	----------	80-100
		28	18	10-30	----------	70-100
Black Eye cowpeas	72 hours at 0.5° to 5°	21	65	----------	----------	----------
		28	50	----------	----------	----------
Delfos cotton	24 hours at 2° to 4°	10	21	0-80	21	0-80
		17	10	0-20	10	0-20
Do	48 hours	10	75	60-90	95	90-100
		17	62	10-90	86	40-100
		25	47	10-92	53	5-100
Do	72 hours	10	97	85-100	100	100
		17	96	89-97	100	100
		25	83	65-90	98	90-100
		35	6.6	0-20	9.8	0-30
		58	8.3	4-15	15.3	6-20
Do	96 hours	25	88	70-96	100	100
		35	18.2	0-35	18.1	0-35
		58	62	50-80	71.2	30-100
Do	108 hours	35	30.6	25-50	48	40-60
		58	72.0	70-85	93.7	5-100

THE RELATION OF SALT SOLUTIONS TO CHILLING INJURY

To learn whether the presence of certain salt solutions in the soil would have an influence on the reaction of plants to chilling, the following experiment was conducted.

Forty cubic centimeters of one-twentieth molecular solutions of calcium chloride, calcium nitrate, potassium chloride, potassium nitrate, sodium chloride, and sodium nitrate were applied to peanut, cowpea, maize, and cotton plants for six consecutive days prior to chilling, previous to which they received no water for 48 hours. To the control plants an equal amount of tap water was applied for the same length of time. For the salt solutions tap water was also used. The plants were chilled when 3 weeks old unless otherwise stated. To find out whether base exchange had a possible bearing on the influence of these salts a part of the maize plants were grown in quartz sand with potassium nitrate, calcium nitrate, and tap water. The influence of these solutions on the retardation or hastening of the ill effects of chilling proved very interesting. The results are given in Tables 8 to 12.

TABLE 8.—*The effect of various salt solutions on the reaction of maize to chilling*

BLOODY BUTCHER CORN (4 WEEKS OLD)

Salt solution used	Percentage injury				Height of plants			
	48 hours after chilling		2 weeks after chilling		Plants chilled		Plants not chilled	
	Average per plant	Range	Average per plant	Range	Average per plant	Range	Average per plant	Range
Chilled 72 hours at 0.5° to 5° C.:					*Inches*	*Inches*	*Inches*	*Inches*
Water	51	45-60	26	20- 30	18.4	18-19		
KNO_3	12	10-15	0	0- 0	22.3	22-23		
$Ca(NO_3)_2$	4	3- 8	11	0- 20	25.0	25-25		
$NaNO_3$	11	10-15	19	15- 30	19.0	16-21		
Chilled 108 hours at 0.5° to 5° C.:								
Water	20	20-20	78	45-100	17.5		23.9	23.5-24
KNO_3	42	30-60	61	35-100	20.5			
$Ca(NO_3)_2$	46	30-60	86	35-100	20.0			
$NaNO_3$	40	40-40	95	75-100	11.0			

KANSAS SUNFLOWER CORN F_1 HYBRID (2 WEEKS OLD)

Salt solution used	Average per plant	Range	Average per plant	Range	Average per plant	Range	Average per plant	Range
Chilled 36 hours at 2° to 4° C.:								
Water			50	50- 50	10	10-10	11.0	11-11
KNO_3			36	30- 40	7.9	7- 8	11.5	11-12
KCl			45.5	45- 48	8.9	8- 9.5	12.0	12-12
CaCl2			60	60- 60	9.0	9- 9	11.0	11-11
$Ca(NO_3)_2$			70	70- 70	8.3	7.5-10	12.0	11-13
NaCl			65	65- 65	9.7	9.5-10	10.0	8-12
$NaNO_3$			84	80- 90	8.0	6- 9	12.0	12-12
Chilled 60 hours at 2° to 4° C.:								
Water			50.0	50- 50				
KNO_3			29.0	25- 45				
KCl			40.0	35- 45				
$CaCl_2$			57.0	57- 57				
$Ca(NO_3)_2$			64.7	61- 68				
NaCl			77.8	70- 90				
$NaNO_3$			85.0	80- 90				

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TABLE 9.—*Effect of salt solutions on the reaction of Midland Yellow Dent maize to chilling*

[2-week-old plants grown in quartz sand, chilled 72 hours at 2° to 4° C.]

Salt solution used	Percentage injury after 1 week	
	Average	Range
Water	26.5	20–30
KNO_3	22.2	0–50
$Ca(NO_3)_2$	74.3	70–85

TABLE 10.—*The effect of salt solutions on the reaction of Early Buff cowpeas to chilling*

[Plants 3 weeks old; chilled at 2° to 4° C.]

Salt solution used	Percentage injury				Height of plants 3 weeks after chilling			
	24 hours after chilling		2 weeks after chilling		Plants chilled		Plants not chilled	
	Average per plant	Range	Average per plant	Range	Average per plant	Range	Average per plant	Range
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Inches*	*Inches*	*Inches*	*Inches*
Chilled 24 hours:								
Water	5	0–15	20	15–25	10.1	9–11	13.2	12–14
KNO_3	13	10–15	12	10–15	10.2	9–12	11.5	10–13
KCl	45	45–45	17	15–20	11.0	9–12	10.0	10–10
$CaCl_2$	32	10–40	37	35–40	10.0	9.5–10.5	12.4	10–14
$Ca(NO_3)_2$	49	35–55	32	30–35	10–2	9–13	12.2	11–13
NaCl	40	25–55	42	40–45	10.1	9.5–11	10.5	9.5–11
$NaNO_3$	50	50–50	50	45–55	10.1	9.5–11	12.2	11–13
Chilled 36 hours:								
Water	40	40–40	40	40–40	8.5	7–10	13.2	12–14
KNO_3	40	40–40	50	50–50	8.3	7.5–10	11.5	10–13
KCl	50	50–50	40	40–40	8.5	8–10	10.0	10–10
$CaCl_2$	56	50–60	70	70–70	8.4	7–10	12.4	10–14
$Ca(NO_3)_2$	50	50–50	60	60–60	9.0	8–10	12.2	11–13
NaCl	60	60–60	75	75–75	7.7	7–8.5	10.5	9.5–11
$NaNO_3$	65	65–65	85	85–85	7.9	7–8.5	12.2	11–13

TABLE 11.—*The effect of salt solutions on the reaction of Spanish peanuts to chilling*

Salt solution used	Average percentage injury				Average height of plants	
	24 hours after chilling	1 week after chilling	2 weeks after chilling		2 weeks after chilling	
			Entire plant	Roots only	Chilled	Not chilled
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Inches*	*Inches*
Chilled 60 hours 2° to 4° C.:						
Water	0	25	40	60	4.5	5.5
KNO_3	0	50	20	30	4.0	7.0
KCl	0	18	30	40	3.5	7.5
$CaCl_2$	0	18	50	50	3.5	7.5
$Ca(NO_3)_2$	0	29	75	75	4.5	6.0
NaCl	0	65	60	55	3.5	7.0
$NaNO_3$	0	75	80	80	4.0	7.0
Chilled 84 hours 2° to 4° C.:						
Water	0	75				
KNO_3	0	75				
KCl	0	80				
$CaCl_2$	0	90				
$Ca(NO_3)$	0	90				
NaCl	0	80				
$NaNO_3$	0	90				

TABLE 12.—*The effect of salt solutions on the reaction of cotton to chilling*

Salt solution used	Percentage injury			Height of plant		
	24 hours after chilling		2 weeks after chilling	Chilled		Not chilled
	Average per plant	Range	Average per plant	Average per plant	Range	Average
WESTEX COTTON (4 WEEKS OLD)						
Chilled 48 hours at 0.5° to 5.0° C.:	*Per cent*	*Per cent*	*Per cent*	*Inches*	*Inches*	*Inches*
Water	17.0	15–40	20	11.4	10.5–12.5	----------
KNO_3	24.0	10–35	10	11.6	11 –12.5	----------
$Ca(NO_3)^2$	35.0	5–70	20	11.8	11 –11.5	----------
$NaNO_3$	22.0	10–30	30	10.0	9 –11	----------
DELFOS COTTON (3 WEEKS OLD)						
Chilled 18 hours at 2.4° to 4.0° C.:						
Water	11.8	0–25	20	6.3	----------	6.5
KNO_3	17.0	0–25	10	6.3	----------	6.6
$Ca(NO_3)^2$	37.0	30–50	20	6.0	----------	6.0
$NaNO_3$	26.0	15–30	25	6.8	----------	6.5

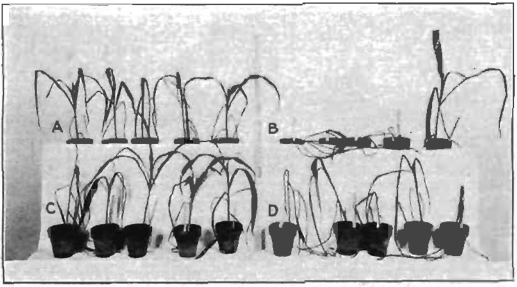

FIG. 8.—Injury to 4-week-old Midland Yellow Dent maize when chilled at 0.5° to 5° C. for 108 hours (severe) after being treated with one-twentieth molecular solution of $Ca(NO_3)^2$ (A), $NaNO_3$ (B), KNO_3 (C) and tap water (D)

The least injury occurred where potassium nitrate had been applied; next in protective effect was potassium chloride. Next in order came those plants that received an equal amount of water, calcium chloride, calcium nitrate, and sodium chloride, while those that had sodium nitrate were injured most, in several instances fatally. (Fig. 8.)

It seems that the potassium cation assisted the plants to withstand chilling, whereas calcium appeared in several instances to cause more injury than that perceptible on the control plants. Sodium had a decidedly deleterious effect. As for the anions less injury occurred where chlorides had been used than with the corresponding nitrates.

The maize plants grown in quartz sand showed that the salt applied rather than a liberated base was responsible for the difference

in injury. In this instance the plants treated with potassium nitrate showed slightly less injury than the control plants, while plants that had received calcium nitrate were nearly killed.

The different reactions brought out by the salt solutions may have been due to the effect of the individual salts on the constitution of the plants. As far as is known, all the cations and anions used, including sodium, are taken up by plants. Especially interesting is the fact that potassium assisted the plants to endure chilling, when it is considered that potassium fertilizers are recommended for application to fields where mazie suffers from root rot. In this experiment, those plants that were treated with sodium nitrate, and were incidentally the most severely injured, were the only ones to show outward indications of the presence of a fusarium similar to that causing maize root rot.

CHILLING INJURIES IN NATURE

Observations made at Colby and Fort Hays experiment stations, and in the Union of South Africa prove that the inimical effects of chilling in nature are in no way different from those artificially produced.

On June 19, 1927, at Colby, Kans., cotton plants 1 month old were observed to have been injured and many killed by "frost." But for at least four weeks prior to this date no frost had been recorded. The weather records, however, showed that the maximum temperatures for June 13 and 14 had been 49° and 50° F., respectively, while the minimum for June 12, 13, and 14 were 45°, 43° and 45° F. respectively. In terms of degrees centrigrade there had been at least a 36-hour period during which the temperature did not go above 10°. The last three days mentioned were cloudy and misty and there was a daily precipitation of 0.6, 0.2, and 0.1 inch, respectively, during that time. The cold and wet weather during these three days was undoubtedly responsible for the injury noted above.

At Fort Hays, Kans., Faris bands in more exaggerated form than those artificially produced were observed on sorghums, maize, and Sudan grass on October 1, 1927. They were particularly noticeable on the leaves of young suckers or the secondary growth of exposed plat corners and on young volunteer plants. A cold and misty period from September 25 to 27 was held responsible for the abnormalities observed. The maximum temperatures for the 26th and 27th were 43° and 45° F., respectively, while the minimum for the 25th, 26th, 27th, and 28th were 42°, 36°, 37°, and 34° F., respectively. In terms of degrees centigrade, during the 26th and 27th there was a 36-hour period when the temperature did not go above 7°.

After the experiments reported here had been completed, both the chilled and the other plants in the greenhouse showed characteristic chilling injury during a cold period from the 17th to the 22d of September. During this period the temperature remained below 50° F. (10° C.) for 13 hours immediately before and after midnight of September 20. The minimum reached was 39° F. (3.89° C.). Normal 3-week-old cowpea plants three days after this chilling behaved no differently than those that had been artificially chilled for 12 hours at 5° to 10° C.

A. R. Saunders reported that during a cold spell in November, 1927, at the experiment station at Potchefstroom, Union of South Africa early-planted maize and amber cane exhibited prominent Faris bands.[4]

[4] Private correspondence; letter of November 26, 1927.

GENERAL DISCUSSION

The investigations reported herein point definitely to the fact that chilling initiates certain changes in crop plants which have a detrimental influence on them. The results thus agree almost completely with those obtained by Molisch (*12*), Faris (*5*), and Pantanelli (*15, 16, 17*). It is of further interest to note that most of the characteristic abnormalities observed by Molisch were noted also in the present study of crop plants and could be described in more or less the same way as recorded by him.

The relative sensitiveness of the plants included in this investigation suggests that their distribution may be dependent quite as much upon their ability to endure chilling as upon other climatic factors, such as total heat units, mean temperatures, frost-free periods, etc., which ordinarilly receive much more attention. Thus plants that proved most sensitive to chilling are the staple crops of the subtropics, such as rice, velvet beans, cotton, and peanuts, while the hardier ones are extensively grown in temperate regions, as, for example, maize, sorghums, watermelons and pumpkins. The most hardy have a very wide distribution, but are essentially northern annuals, such as soy beans, buckwheat, flax, and sunflowers.

That varietal distribution may also be dependent upon chilling injury is definitely suggested by the difference in hardiness of certain varieties of cotton, peanuts, cowpeas, and soy beans.

What the ultimate cause of the deleterious influences that follow chilling may be is a matter requiring further investigation. It is well known that physiological processes are definitely limited by temperature, some much more than others. Puriewitsch (*18*), according to Palladin (*14*), found that the respiratory ratio is at a minimum from 2° to 4° C.; that is, at this temperature oxygen absorption proceeds much faster than the elimination of carbon dioxide. Oxidation in the cells is therefore not complete and toxic substances may be formed. It is therefore conceivable or even probable that low temperatures upset the sensitive balance between the various processes with the result that harmful products accumulate in the plant cells. This seems to have been Molisch's view (*12*).

In the investigations reported in this paper greater injury was observed on plants growing in saturated, and therefore in an oxygen-deficient soil, than in dry soil. This conceivably might accentuate the relation noted above.

The carbohydrate nitrogen ratio in plants may also be a very important factor in determining the resistance to chilling. Pantanelli concluded from his experiments that those plants which retained the most sugar are able to withstand cold best, because at low temperatures carbohydrates are used in respiratory combustion. He pointed out further that as long as a cell has sugar at its disposal it does not consume albumens and when the sugar is exhausted autodigestion of the proteins takes place. Since nitrogen is a limiting factor in growth, it follows that the more nitrogen there is present in the plant the more carbohydrates are used up in building new tissue. According to the work of Fischer (*6*) and Kraus and Kraybill (*9*) a rapid vegetative growth is characterized by a high nitrogen carbohydrate ratio. Hence those plants making the most active growth and thus using considerable carbohydrates are likely to have the

least in reserve for respiratory combustion during a cold period. This hypothesis is substantiated by the fact that in general where nitrates were applied the greatest amount of injury took place. The single exception, with potassium nitrate, is easily explained by the well-known fact that potassium functions especially in photosynthesis and in the movement of carbohydrates within the plant, and that it tends to counteract the ill effects of too much nitrogen.

Nelson (*13*) suggests from his own and the work of Armstrong (*1*) that at low temperatures there is a liberation or accumulation of some toxic fragments resulting from the mixing of hydrolytic enzymes and glucoside. The protoplasm being unable to function properly at the low temperatures can not throw off these toxic compounds in the normal manner.

That certain plants die from drought after their roots have been injured to a greater or lesser extent is most probable. In the present study this was found to be true with peanuts. The plants did not show injury or perish until the roots had been injured to such an extent as to be unable to supply the top growth with an adequate amount of water.

In conclusion it must be pointed out that the chilling of plants should not be considered from the point of air temperatures alone; soil temperatures warrant as much consideration. In spring soil temperatures are considerably lower than air temperatures and the former fluctuate far less than the latter. The top growth of the plants may experience a chill first, and when the air has warmed up again the roots may still be in very cold soil. The chilling period to which the plant as a whole is subjected may thus be longer than that indicated by either soil or air temperatures separately.

SUMMARY AND CONCLUSIONS

The results of the experimental work reported in this paper support the following conclusions:

In most summer crop plants constitutional disturbances having a serious influence on them are called into effect when the plants are subjected to chilling, even though the temperature does not go as low as 0°C.

The outstanding result of the investigation was the very evident effects of chilling on certain plants and the high degree of resistance in others. The duration and the intensity of the cold were important factors in determining the nature of the reactions. An exposure of 24 and 36 hours at 0.5 to 5.0° C. was fatal to rice, velvet beans, and cotton. With the same exposure cowpeas were completely defoliated and only straggly plants were produced. Peanuts, Sudan grass, and Teff grass exposed to chilling temperatures for 48 hours appeared at first to be uninjured, but died in the course of about two weeks; at the same exposure maize, sorghums, watermelons, and pumpkins were slightly injured. Soy beans, potatoes, buckwheat, Tepary beans, tomatoes, and flax proved to be exceedingly hardy, it being possible to expose them to chilling temperatures for 84 to 96 hours without injury. At 5° to 10° C. velvet beans proved to be hardier than Whippoorwill cowpeas. At this temperature cowpeas were injured when exposed for 24 hours, velvet beans and cotton when exposed 60 hours.

Marked differences in relative hardiness between varieties was observed. Trice cotton, which is grown near the northern limit of

the Cotton Belt in North Carolina, proved more hardy than Delfos from Mississippi, while Westex, a variety specially bred for Texas conditions, was considerably more tender than either of the foregoing. Whippoorwill cowpeas from Georgia became chlorotic when chilled for 12 hours at 5° to 10° C., whereas Early Buff from Kansas did not assume this condition until chilled for 36 hours at 0.5° to 3.0°. Virginia Bunch peanuts were hardier than Spanish and the latter hardier than Valencia. Early Prolific rice was more sensitive than Honduras, and Manchu soy beans were hardier than Virginia. The relative hardiness of the different plants indicates that their distribution from subtropical to temperate regions may be dependent to some extent upon their ability to endure chilling.

A point of considerable interest was the characteristic reactions of different species, not only with respect to differences in degree of injury, but also with respect to the nature of the injury. Cowpeas and rice leaves became chlorotic 24 to 48 hours after chilling, while cotton and soy beans showed brown and white spots, respectively, and the grasses in general showed white bands across the leaves similar to those described by Faris in sugar cane. Lesions were observed on field crops at Hays and Colby and at Manhattan on greenhouse plants when the air temperature remained between 33° and 50° F. for one to three days.

Cowpeas, peanuts, maize, and velvet beans were far more severely injured in wet than in dry soil. Soy beans behaved in the contrary manner, although the injury in the latter case was slight and may possibly have been due to drought.

In experiments with cowpeas, cotton, and velvet beans, young plants suffered materially more than old plants treated in a similar manner.

Tap water solutions of various salts were applied to the plants to ascertain whether any salts would act protectively against plant injury by chilling. Equal volumes of tap water were applied to control plants. Potassium nitrate was found to afford considerable protection, with potassium chloride standing next in order. Then came the control lot, and then, in descending order of effectiveness, the lots to which calcium chloride, calcium nitrate, sodium chloride, and sodium nitrate were applied. Sodium nitrate and sodium chloride were in some cases fatally injurious when chilling was continued for from 36 to 60 hours, the former salt being the more injurious.

It appears that the potassium cation assisted the plants to withstand chilling better than if they were merely moistened with tap water. Calcium, however, seemed to exert no such protective influence, as plants receiving it were in some instances injured more by chilling than the control plants. Sodium had a decidedly deleterious effect. As for the anions, less injury seemed to occur in the presence of the chlorides than when nitrates were used, except in the case of potassium nitrate.

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INJURY TO ONIONS AND FRUITS CAUSED BY EXPOSURE TO AMMONIA [1]

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INTRODUCTION

For several years the pathologists of the Bureau of Plant Industry associated with the food-products inspection service of the Bureau of Agricultural Economics have been asked from time to time to explain the cause of certain brownish and greenish black discolorations of fruits and onions. In most cases the products in question were free from disease and appeared perfectly normal in every respect except color. The discoloration of onions has been observed more frequently than that of any other product, but similar discolorations of apples, pears, bananas, and peaches have been seen.

RESULTS OF INVESTIGATIONS

ONIONS

The discoloration of onions was investigated first. The senior writer was asked to decide whether a certain lot of onions had been injured in storage. The grower claimed that the onions were bright yellow, clean, and in good condition when they were placed in cold storage, but that when they were taken out to be placed upon the market the outer dry scales were brownish black, as if they had been scorched or burned. Careful examination of the samples furnished showed that the fleshy scales were in good condition and normal in color. No indication of freezing injury or parasitic disease could be found, consequently it became necessary to look for other causes of the discoloration. The fact that the injury in this particular lot of onions appeared to be directly associated with some unusual storage condition suggested the possibility that escaping ammonia from the cold-storage system might have been the causal agent.

In order to study the effect of ammonia fumes on onions, several white onions were placed on wire racks in two 5-liter jars, 15 c. c. of ammonia (specific gravity 0.90) was poured into each jar, and a glass plate was sealed over the top of each with vaseline. These experiments were identical except that the onions in one jar were moistened with water, while those in the other were dry. Within an hour the moistened onions were slightly yellowish, while the dry ones were only faintly greenish yellow along the broken edges of the dry scales. These onions were kept under observation in the ammonia fumes for one month. At the end of this period the moistened onions were

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[2] Contribution from the office of vegetable and forage diseases, Bureau of Plant Industry, U. S. Department of Agriculture; the department of botany, University of Chicago, cooperating. The writers wish to express thanks to T. F. Young, of the department of chemistry, and G. K. K. Link, of the department of botany, University of Chicago, for suggestions.

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"chamois"[3] to light brown and the dry ones had a metallic sheen and were "cinnamon buff" in color. This prolonged exposure caused the death of all tissues and a yellow to mustard-yellow discoloration throughout all bulbs. However, all the badly discolored onions observed on the market were yellow, brown, or red varieties.

Yellow onions, when treated like the white ones, immediately began to turn greenish yellow and then brown. After an exposure of one hour all of them were dark, some deep sepia brown, and others almost black. The moistened onions darkened first, but there was little difference between the two lots at the end of an hour. They were kept under observation in the ammonia fumes for three days. At the end of this period all of them were dark brown to metallic black. The brownish black discoloration was confined to the pigment-bearing outer dry scales, but there was also much injury to the two or three outer layers of fleshy scales, which were soft, flabby, and greenish yellow.

These preliminary experiments showed that strong ammonia injures onions seriously, and indicated that ammonia had probably caused the discoloration of the storage onions that led to this investigation. The results served as a basis for further work on the discoloration of onions and on certain similar discolorations of fruits.

Additional tests were made in the laboratory for the purpose of checking preliminary experiments and observations made on the market and of studying the progressive action of known concentrations of ammonia on the plant tissues and the subsequent reactions of the specimens. No attempt was made to determine the minimum quantity of ammonia that would cause injury. Although no critically quantitative tests were made, the percentage of ammonia in the air was determined. This was done in two ways: First, by means of a gas burette;[4] and second, by titrating solutions of ammonium hydroxide and calculating from the molar concentration the percentage of ammonia in the solution. Two strengths of ammonium hydroxide were used. The normality, the percentage of ammonia, the resulting vapor pressure[5] of these, and the percentage of ammonia in the air are shown in Table 1. The tests with this method were carried out at a temperature of 70° F. and a barometric pressure of 750.7 mm. Equal quantities of each of the solutions were placed in dishes under bell jars; then, after 10 minutes, to insure diffusion of the ammonia through the air, the specimens were supported on wire baskets over the solutions and the observations were begun.

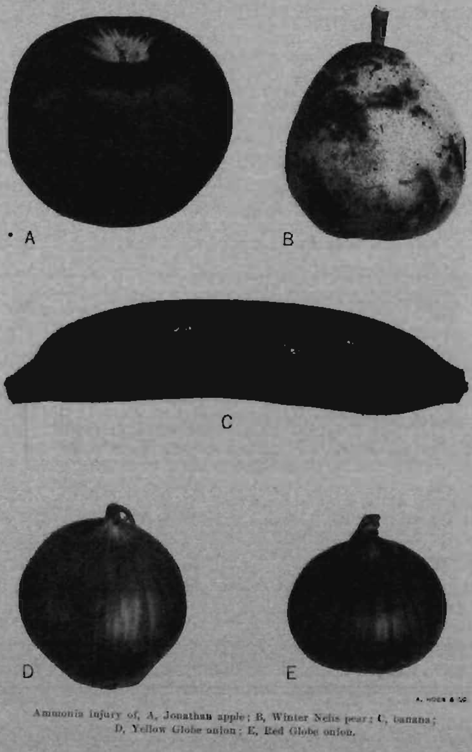
TABLE 1.—*Significant figures in the calculation of percentages of ammonia in the air from solutions of ammonium hydroxide*

Normality of solution	Ammonia in solution (per cent by weight)	Vapor pressure of ammonia (mm. mercury)	Ammonia in air (per cent by volume)
2.68	4.56	30.40	4.18
11.30	19.25	212.90	29.29

[3] Throughout this paper all quoted color names are from the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

[4] *All determinations were made by means of the gas burette except the experiments at 70° F. with 4.2 and 29.3 per cent ammonia.*

[5] LANDOLT, H. H. LANDOLT-BÖRNSTEIN PHYSIKALISCH-CHEMISCHE TABELLEN. Ed. 5, p. 1297. Berlin. 1923.



Ammonia injury of, A, Jonathan apple; B, Winter Nelis pear; C, banana; D, Yellow Globe onion; E, Red Globe onion.

In Table 2 are given the results of representative experiments conducted at a temperature of 70° F. and at relative humidities of 30 and 85 per cent, in which yellow and red onions were exposed to known strengths of ammonia for definite periods of time. The color changes are described in each instance. It will be noted that the edges of the dry color-bearing scales began to show a greenish yellow discoloration almost instantly in each of the concentrations of ammonia used. This color change is characteristic and marks the initial reaction which eventually results in various gradations of color from greenish yellow in white onions to brown in yellow ones (pl. 1, D) and deep greenish black in red ones (pl. 1, E), upon prolonged exposure to ammonia. The results of these experiments indicate that a small amount of ammonia (0.8 per cent) in the air will cause in a lesser degree the same discoloration that is produced by a high percentage, but that the rate will be slightly lower. This is especially noticeable with the yellow onions, which as a rule are somewhat slower than the red ones in showing the effect of ammonia. The character and condition of the pigments as well as the degree of pigmentation determine the extent of discoloration possible. In all experiments the most highly colored specimens showed the most injury.

TABLE 2.—*Effect of ammonia on Red Globe and Yellow Globe onions at a temperature of 70° F., and relative humidities of 30 and 85 per cent*

Relative humidity (per cent)	Percentage of ammonia	Duration of exposure	Color changes in—	
			Red onions	Yellow onions
30	0.8	½ minute	Greenish ("citron yellow" to "yellowish citrine") along edges of scales.	No change.
		2 minutes	----------	Yellowish green ("citron yellow") along edges of scales.
		10 minutes	Large blackish green ("dull blackish green") blotches prominent.	Light-bronze ("medal bronze") blotches prominent.
		42 hours	50 per cent of surface blackish green ("dull blackish green").	20 per cent of surface bronze ("medal bronze" to "raw umber").
30	1.0	½ minute	Blackish green ("dull blackish green") blotches prominent.	Edges of scales greenish brown ("dark citrine").
		15 minutes	Large blackish green ("dull blackish green") blotches.	Large bronze ("medal bronze") blotches.
		30 minutes	70 per cent of surface blackish green ("dull blackish green").	Large dark bronze ("medal bronze" to "raw umber") blotches.
		48 hours	90 per cent of surface dark greenish black ("dull greenish black (2)").	75 per cent of surface dark bronze ("raw umber").
85	4.2	½ minute	Blackish green ("dull blackish green") along edges of scales.	Greenish yellow ("yellowish citrine") along edges of scales.
		30 minutes	Large dark blotches of blackish green ("dull blackish green").	Cinnamon brown ("cinnamon rufous") in blotches.
		1½ hours	Most of surface dark greenish black ("dull greenish black (2)").	Large cinnamon-brown to brownish black ("cinnamon-rufous" to "chestnut brown") blotches.
		4½ hours	95 per cent of surface dark greenish black ("dull greenish black (2)").	95 per cent of surface bronze to brownish black ("Chestnut brown" to "Vandyke brown").
85	20.3	½ minute	Blackish green ("dull blackish green") along edges of scales.	Greenish yellow ("yellowish citrine") along edges of scales.
		30 minutes	Most of surface blackish green ("dull blackish green").	Most of surface brown ("cinnamon brown").
		1½ hours	90 per cent of surface dark greenish black ("dull greenish black.")	Most of surface dark brown ("chestnut brown").
		4½ hours	98 per cent of surface dark greenish black to metallic black ("dull greenish black (2)").	98 per cent of surface blackish brown ("Vandyke brown").

Since the discoloration took place so rapidly at 70° F., it seemed advisable to conduct experiments to determine what effect low temperatures would have upon the rate of discoloration. Consequently, several experiments were made with comparatively low percentages of ammonia in a cold-storage room where the temperature was 31.5° and the relative humidity 83 per cent.

The results of typical experiments conducted under low-temperature conditions are shown in Table 3. The characteristic greenish black discoloration of the red onions and the yellowish green and brown of the yellow ones were quite noticeable within a few minutes in each of the concentrations. With 0.8 per cent ammonia the initial color changes took place somewhat more slowly than in the corresponding experiments at 70° F., but marked discoloration was produced in less than an hour. With 3.2 per cent ammonia the greenish discoloration along the broken edges of the dry color-bearing scales was almost instantaneous, prominent blotches being evident within one-half minute. This action is as rapid as that recorded for higher concentrations of ammonia at 70°. In experiments where concentrations as low as 3.2 per cent were used at both 31.5° and 70° no difference in the initial rate of discoloration could be detected. These experiments, together with others of a similar nature that were conducted in cold storage, show, therefore, that low temperatures do not materially influence the rate of discoloration of onions exposed to ammonia.

Humidity is one of the important factors in determining the rate of discoloration, and with concentrations of ammonia lower than 1 per cent some experiments indicate that it may determine whether or not any discoloration will take place. In all experiments where any noticeable variation in air moisture or dryness of the onion scales existed, discoloration was always much more rapid and pronounced under the more humid conditions.

TABLE 3.—*Effect of ammonia on Red Globe and Yellow Globe onions at a temperature of 31.5° F., and a relative humidity of 83 per cent*

Percentage of ammonia	Duration of exposure	Color changes in— Red onions	Color changes in— Yellow onions
0.8	7 minutes	Edges of broken scales yellowish green ("yellowish citrine").	No change.
	43 minutes	10 per cent of surface blackish green ("dark olive" to "dull blackish green").	Edges of broken scales yellowish green ("citron yellow").
	19½ hours	30 per cent of surface blackish green ("dull blackish green").	Yellowish green to greenish brown ("yellowish citrine" to "dark citrine") along edges of scales and 10 per cent of surface.
2.0	5 minutes	Blackish green blotches evident ("dull blackish green").	Greenish brown blotches prominent, ("dark citrine").
	17½ hours	98 per cent of surface dark greenish black ("dull greenish black (2)").	95 per cent of surface dark bronze ("raw umber" to "chestnut brown").
3.2	½ minute	Blackish green ("dull blackish green") blotches prominent.	Greenish brown ("dark citrine") along edges of scales.
	8 minutes	Most of surface blackish green ("dull blackish green").	Most of surface metallic bronze ("raw umber" to "chestnut brown").
	1½ hours	95 per cent of surface dark greenish black to metallic black ("dull greenish black (2)").	90 per cent of surface bronze to brownish black ("chestnut brown" to "Vandyke brown").

Many of the ammonia-injured onions observed on the market resembled onions exposed experimentally at a temperature of 70° F. and a relative humidity of 85 per cent, to 29.3 per cent ammonia for 1½ hours, and 4.2 per cent ammonia for 4½ hours, or at a temperature of 31.5 ° and a relative humidity of 83 per cent to 2.6 per cent ammonia for 17½ hours. The injury at the end of these exposures is to be classed as a blemish, since the color-bearing dry outer scales are usually the only ones affected. Any of the fleshy scales that are exposed by the removal of the dry protective scales turn greenish yellow within the time limits mentioned above, but if the dry scales are intact, there is no injury of the edible portion of the bulbs. Longer exposures are practically certain to cause some discoloration and softening of the fleshy scales. The reactions of the individual bulbs show wide differences in this respect, however, on account of the variations in number, texture, and condition of the protective scales. When onions are exposed to ammonia long enough to cause death of the outer fleshy scales they become greenish yellow, watery, and unmarketable and subject to attack by decay-producing organisms.

APPLES

When well-colored, firm Jonathan apples were exposed to ammonia fumes they quickly became discolored, the injury showing first around the lenticels. After removal from the fumes the apples began to recover their normal color almost at once, and after 12 hours practically all of the coloring had returned and no further change could be noted. The progressive changes while in contact with the ammonia are recorded in Table 4, in which, for convenience, results with other fruits are also included. As just indicated, the changes represent transitory conditions.

After the recovery noted above no change could be observed and whatever discoloration remained was permanent. The appearance of apples that have been exposed to ammonia is characterized chiefly by the discoloration around the lenticels. (Pl. 1, A.) On the blushed side they are surrounded by dark rings, which are generally from 1 to 2 mm. in diameter and darker than the surrounding tissues. These rings are black when they occur on the deep-red areas and green on the yellow-green areas.

Jonathan apples were also exposed to ammonia (0.8 and 1.0 per cent) at a temperature of 70° F. and a relative humidity of 30 per cent. The rate of discoloration was slightly slower than that recorded for the higher percentages of ammonia (Table 4), but in general the results were not sufficiently different to warrant detailed discussion.

The effect of low temperature upon the discoloration of apples was also determined. (Table 5.) The results indicate that lowering the temperature did not appreciably alter the rate of discoloration.

PEARS

In their work on the storage of pears, Overholzer and Latimer[6] describe ammonia injury of pears as follows:

> Pears contain a substance that becomes dark when alkaline and colorless when acid. Ammonia gas gives an alkaline reaction, and since the fumes are quite soluble in fruit juices, they may be absorbed through the lenticellike openings

[6] OVERHOLZER, E. L., and LATIMER, L. P. EFFECT OF AMMONIA FUMES UPON PEARS IN COLD STORAGE. Calif. Agr. Expt. Sta. Rpt. 1922/23: 201-203, illus. 1923.

or "dots" of the epidermis sufficiently to bring about an alkaline condition in local areas.

The oxidation processes in pears also proceed more rapidly in a slightly alkaline medium than in the acid tissues of the fruit. Furthermore, the concentration of ammonia may become sufficiently great to act deleteriously by increasing the permeability of the cells and thus disorganizing the protoplasm and permitting the mixing of the oxidizing enzyme and substrate. Hence, fruit subjected to ammonia gas or fumes may also darken by oxidation, but the discoloration thus produced is apparently distinct from that effected immediately by alkalinity. The two colors, however, are inseparable upon the basis of appearance.

TABLE 4.—*Effect of ammonia on Jonathan apples, Bartlett pears, bananas, and peaches at a temperature of 70° F., and at relative humidity of 85 per cent*

Percentage of ammonia	Duration of exposure	Color changes
		APPLES
4.2	1 minute	Lenticels browned.
	2 minutes	Do.
	6 minutes	Few dark rings around lenticels.
	11 minutes	Few greenish rings around lenticels on yellow side of apple.
	19 minutes	Slight darkening of red areas.
	30 minutes	A few additional rings and streaks of reddish purple ("dark Corinthian purple") through purplish red ("burnt lake"). No discoloration of unblushed areas.
29.3	1 minute	Lenticels browned.
	2 minutes	Dark rings around a few lenticels.
	6 minutes	Rings more numerous, larger and darker.
	11 minutes	Lenticels show white specks in center. Unblushed areas changing to yellowish green.
	19 minutes	Unblushed areas yellowish green. Blushed areas dark, nearly black.
	30 minutes	Deeper red has turned to glossy black that shades through brown and red into greenish yellow ("deep colonial buff") of unblushed areas.
		PEARS
4.2	2 minutes	Lenticels rather prominent, greenish brown.
	7 minutes	Lenticels rather prominent, greenish brown. Minute drops of moisture on the surface.
	12 minutes	Do.
	17 minutes	No change in number of spots but spots changed to dull red ("brick red").
	20 minutes	Surface generally moist, spots reddish brown, no coalescing visible.
29.3	2 minutes	Lenticels prominent dull red ("brick red").
	7 minutes	Increase in size and number of red lenticels, surface quite moist.
	12 minutes	Spots enlarged due to spreading of discoloration from lenticels.
	17 minutes	Spots coalescing rapidly.
	20 minutes	Surface wet. Ground color light brown ("Sanford's brown") with reddish brown ("burnt sienna") blotches.
		BANANAS
4.2	12 minutes	Few small yellowish-brown dots on sides.
	28 minutes	10 per cent of surface spotted as above.
	1 hour	25 per cent of surface brown; ribs dark brown to black. Ends black, some reddish brown blotches.
29.3	4½ minutes	Minute yellowish brown dots generally distributed.
	10 minutes	Spots darker brown, 20 per cent discoloration. Dead-ripe appearance.
	24 minutes	Ribs solid black. Minute drops of moisture on surface.
	33 minutes	Generally dark brown with black ribs and ends.
	1 hour	Uniformly brownish black ("Mars brown" to "clove brown").
		PEACHES
4.2	1 minute	Minute greenish-black specks.
	3 minutes	Red color of peach gone. Many greenish black specks.
	23 minutes	60 per cent of surface dull yellowish green ("olive lake" and "buffy citrine").
	1 hour 40 minutes.	Almost solid dull yellowish green ("olive lake").
29.3	1 minute	Minute greenish black specks.
	23 minutes	Dull yellowish green ("olive lake" and "buffy citrine").
	1 hour 10 minutes.	Darker with a reddish cast. Some moisture on surface.
	1 hour 40 minutes.	As above with large reddish brown blotches and large drops of moisture, on surface.

TABLE 5.—*Effect of ammonia on Jonathan apples, Anjou pears, and bananas at a temperature of 31.5° F., and a relative humidity of 83 per cent*

APPLES

Per centage of ammonia	Duration of exposure	Color changes
0.8	9 minutes	Few lenticels show dark borders and white centers.
	43 minutes	Many lenticels show heavy black borders and white centers.
	19½ hours	Lenticels prominent as white dots, but few black borders remain.
2.6	5 minutes	Lenticels prominent, black borders with white centers.
	17½ hours	Lenticels white surrounded by black rings 1.5 to 4 mm. in diameter.
3.2	½ minute	Lenticels prominent, few black borders.
	8 minutes	Lenticels prominent with black rings, discoloration spreading between rings.
	1½ hours	90 per cent of surface discolored. Lenticels with white centers and black borders.

PEARS

Per centage of ammonia	Duration of exposure	Color changes
0.8	22 minutes	Lenticels slightly watery. Light reddish blotches appearing.
	43 minutes	Lenticels more moist and reddish blotches more prominent.
	19½ hours	Lenticels watery, blotches as above.
2.6	5 minutes	Few slightly reddish blotches.
	17½ hours	Few slightly reddish blotches. Few lenticels with reddish borders, fruit deeper yellow and more mature looking.
3.2	2 minutes	Few light reddish blotches.
	8 minutes	Few reddish brown blotches.
	1½ hours	Reddish brown blotches larger. Ruptures in skin reddish brown.

BANANAS

Per centage of ammonia	Duration of exposure	Color changes
0.8	9 minutes	Few brownish blotches.
	43 minutes	Brownish discoloration general.
	19½ hours	Many black blotches, dead-ripe appearance.
2.6	5 minutes	General color light brown, blotchy.
	17½ hours	Solid black, much moisture on surface.
3.2	½ minute	Brownish blotches general.
	8 minutes	Black blotches on brown, dead-ripe appearance.
	1½ hours	95 per cent of surface black, much moisture on surface.

A further observation included in the same paper was that the lenticels exhibit crimson-colored rings, which quickly become black. The depth of penetration was from three to five cells except under prolonged exposure. Exposure of five to seven days caused darkening one-half inch deep and a darkening of the vascular bundles. It was observed also that green pears are more seriously injured than ripe ones, and that increasing humidity increases the action of the ammonia.

In the present work no prolonged tests were made, the concentrations of ammonia used being high enough to show marked results in a very short time.

Bartlett pears that had not yet begun to become mellow were used. As shown in Table 4, changes in the color of the epidermis appeared in less than two minutes after they were subjected to ammonia. The injury appeared first around lenticels and abrasions, the ammonia apparently having no effect upon the color of the epidermal cells except those next to an opening. The coalescing of the spots, which occurs later, appears to be due to the diffusion of ammonia through the subepidermal tissues and not to any absorption through

the cutinized, unbroken epidermis. It is also interesting to note that the pears remained reddish brown as long as they were exposed to the ammonia. When removed and held in the air at room temperature, the reddish brown color gradually turned darker and also spread until at least 50 per cent of the pear surface was black. (Pl. 1, B.) This change required about four hours. The pears exposed to the stronger ammonia were blotched brown and reddish brown when removed from the bell jar and became solid black within four hours. Penetration in the pears exposed to the less concentrated ammonia was confined to a depth of four or five cells below the epidermis; in the pears held in the more concentrated ammonia it reached greater depths. When fruit was exposed to the ammonia for 20 minutes, the vascular tissues were brown to a depth of 1.5 mm., and when held for a longer period the browning extended 8 to 10 mm. below the epidermis. These examinations were made 24 hours after the pears were removed from the ammonia.

Winter Nelis pears were exposed to ammonia (0.8 and 1.0 per cent) at a temperature of 70° F. and a relative humidity of 30 per cent. The rate of discoloration was slightly slower than that recorded for the higher percentages of ammonia (Table 4), but the results of these tests did not differ significantly from the others.

Anjou pears were subjected to ammonia fumes at low temperature. (Table 5.) The results indicate that lowering the temperature did not appreciably alter the rate of discoloration. Anjou pears are less susceptible than Winter Nelis.

BANANAS

The bananas used were light yellow with green ends and were quite firm. On exposure to ammonia, the color changes took place quickly in the weak ammonia, but progressed more rapidly in the more concentrated solution. (Table 4.) Even a short exposure caused a darkening of the peel, which persisted after removal from the ammonia chamber and gave the banana a dead-ripe appearance. (Pl. 1, C.)

Bananas were also exposed to ammonia fumes in the same way as Winter Nelis and Anjou pears. (Table 5.) The results were not sufficiently different to warrant more detailed discussion.

PEACHES

In addition to the changes in color (Table 4), peaches shriveled rapidly when removed from the ammonia. Three days after treatment they were dried and wrinkled and resembled mummies produced by brown rot. The flesh was brown throughout, indicating a more rapid and deeper penetration of the gas than was found in the other fruits studied.

DISCUSSION

As pointed out by Wheldale,[7] the reaction of the anthocyanins with alkalies and acids has been a matter of interest to investigators for many years. Although in this paper no attempt has been made to differentiate the various color pigments in the fruits and onions under consideration, it seems safe to assume that they are chiefly anthocyanins and anthoxanthins. The term anthocyanin is used

[7] WHELDALE, M. THE ANTHOCYANIN PIGMENTS OF PLANTS. 318 p Cambridge. 1916.

here in a collective sense, to include the soluble pigments of blue, red, and violet tints of plants and plant products, while anthoxanthin [8] is used in a like manner to include the yellow and brown pigments. In his work on the relation of onion pigments to disease, Walker [9] states that the red and yellow pigments are solutes in the cell sap of the epidermal layer of the colored scales. He also mentions the fact that the pigmented cells of the yellowish varieties turn deep brownish yellow when treated with alkalies, a reaction typical of the flavones, and those of the red varieties turn pink in acid and green in alkaline solutions, a characteristic reaction of the anthocyanins. The anthocyanins are normally in solution in the cell sap, but sometimes the concentration becomes so great that the pigments separate out in a crystalline or an amorphous condition. This amorphous condition is generally found in the dry colored scales of cured onions that are placed upon the market. As the color-bearing scales die, their cell walls are frequently stained by exosmosis of the coloring matter, and it is along the broken edges of the scales where these cells are exposed that the first color reactions take place when onions are exposed to ammonia.

With the possible exception of bananas, the surface discoloration of colored onions and fruits by ammonia is, therefore, to be attributed primarily to the action of the gas on the anthocyanins and anthoxanthins, while the end results are somewhat modified by the death of cells and by color changes incident to oxidation. The blackening of bananas occurs so soon after the initial discoloration that it is difficult to distinguish between the two reactions.

As indicated in the experiments described, ammonia injury may be merely a blemish, as in the discoloration of the outer dry color-bearing scales of the onion and the darkening of the lenticels of the apple and the pear, or it may be serious as in the softening and discoloring of the edible portions of onions and in the browning and softening of pears, bananas, and peaches. The strength of the ammonia fumes, the relative humidity, and the duration of the exposure determine the extent of the injury. Ordinarily, the injury produced by ammonia can be distinguished from other discolorations found in fruits and onions by the typical alkaline reaction produced with the pigments in the color-bearing surface tissues, although occasionally somewhat similar discolorations may be caused by other unfavorable conditions or by disease.

SUMMARY

Brownish and greenish black discolorations of onions and fruits that appear normal in every respect except color have frequently been observed on the Chicago market. Discolored onions have been found most often, but discolored apples, pears, peaches, and bananas also have been noted. The absence of a causal organism and the consistent association of the injury with storage indicated that the discoloration had resulted from exposure of the products to a uniformly distributed deleterious substance while held in storage.

[8] WILLSTÄTTER, R., and EVEREST, A. E. UNTERSUCHUNGEN ÜBER DIE ANTHOCYANE. Liebigs Ann. Chem. 401: 189-232. 1913.

[9] WALKER, J. C. DISEASE RESISTANCE TO ONION SMUDGE. Jour. Agr. Research 24: 1019-1040, illus. 1923.

This type of injury was duplicated in the laboratory when onions and fruits were exposed to ammonia (0.8 to 29.3 per cent) at 70° F. and relative humidities of 30 and 85 per cent, and in cold storage at 31.5° with 0.8 to 3.2 per cent ammonia and a relative humidity of 83 per cent. The extent of the injury was determined by the percentage of ammonia in the air, the relative humidity, and the duration of the exposure. Variations in temperature did not greatly influence the rate or extent of discoloration.

It was demonstrated that the yellow, brown, and red pigment-bearing tissues become brownish to dark brown and in some cases greenish black when exposed to ammonia. The injury may be merely a blemish, as in the discoloration of the outer dry color-bearing scales of the onion and darkening of the lenticels of the apple and pear, or it may be serious, as in the softening and discoloring of onions and in the browning and softening of pears, bananas, and peaches.

THE INFLUENCE OF OXYGEN AND CARBON DIOXIDE ON THE GROWTH OF OPHIOBOLUS GRAMINIS IN PURE CULTURE[1]

By HURLEY FELLOWS

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INTRODUCTION

During the last few years Sewell and Melchers[2] have made some interesting observations on the destructiveness of take-all (*Ophiobolus graminis*) in the experimental wheat plots of the Kansas Agricultural Experiment Station at Manhattan, Kans. They noted that different kinds of cultivation in the plots apparently caused differences in the destructiveness of the take-all disease.

In inoculating wheat plants with *Ophiobolus graminis* the writer observed that sometimes, for no apparent reason, infection failed to develop. These inoculations were made and the inoculated plants kept in a greenhouse where the temperature was controlled and kept constantly favorable for infection. The soil moisture also was kept favorable for infection. Hence variation in the gas content of the soil suggested itself as a possible explanation of the apparent variations in the pathogenicity of the fungus.

Of the environmental factors that affect the development of a parasite in the soil, moisture, temperature, and acidity have received the most careful consideration from pathologists. Thus far little attention has been paid to the influence that changes in the gas content of the soil may exert on the growth and activity of the common soil-inhabiting parasites. As *Ophiobolus graminis* is influenced by different soil moistures and temperatures, which, in turn, affect the gas content of the soil, it was believed that, if the organism should prove sensitive to the variations in soil gases, its increase and activity might be controlled somewhat by any methods of cultivation that varied the gas content of the soil. With this in mind, the writer, in 1925, conducted a series of experiments in which pure cultures of *O. graminis* were grown in air containing different percentages of carbon dioxide and oxygen, respectively.

MATERIALS AND METHODS

The parasite was grown on solid media in Petri dishes and on liquid media in Erlenmeyer flasks. These were uniformly inoculated, each with a small piece of agar with new mycelium of the fungus, and placed in large glass jars closed with ground-glass tops. Through the top of

[1] Received for publication May 22, 1928; issued November, 1928. These investigations were conducted in cooperation with the Kansas Agricultural Experiment Station, Manhattan, Kans., and this paper is No. 274 of the Department of Botany and Plant Pathology.

[2] SEWELL, M. C., and MELCHERS, L. E. THE EFFECT OF ROTATION AND TILLAGE ON FOOTROT OF WHEAT IN KANSAS, 1920–1924. Jour. Amer. Soc. Agron. 16: 768–771, illus. 1924.

each jar three glass tubes were inserted and sealed in. One of these tubes extended to the bottom and served to admit the proper gas mixture that was to surround the cultures within. Another short tube permitted the removal of a small gas sample for analysis. The third tube had on its lower end a small rubber gas bag for keeping the barometric pressure inside the jar equal to that outside. The flask or Petri-dish cultures were placed in the bottom of the jar, which was then tightly sealed. A diagram of the apparatus is shown in Figure 1. The Petri-dish covers were raised slightly by means of small bent wires so placed that the gas in the Petri dishes could diffuse freely with that of the culture jar. The flasks were loosely plugged with cotton for the same reason.

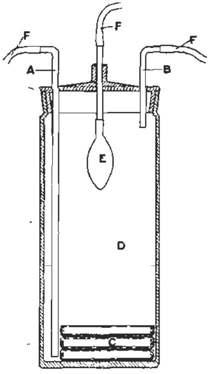

FIG. 1.—Diagram of a specimen jar used as a gas-control culture chamber. A, Intake tube for gas mixtures; B, tube from which gas samples are taken for analysis; C, Petri dishes in which the organism is cultured; D, main portion of chamber in which various gas mixtures are retained; E, rubber gas bag used to keep the barometric pressure inside the jar equal to that on the outside; F, rubber-tube terminals upon which pinch cocks are placed

It was thought possible that the growth of the fungus might differ on different media, especially if one were solid and the other liquid, even though the concentrations of the gases of the surrounding atmosphere were constant. Accordingly, potato-dextrose agar and potato-dextrose decoction were selected as media. The growth measurements were made by weighing the dried mats of mycelium from the liquid medium and by measuring the diameters of the colonies on the agar medium.

Five different respective concentrations each of oxygen and carbon dioxide were used. (Tables 1 and 2.) When the concentration of carbon dioxide was varied that of oxygen was kept as near as possible to the percentage in the air, which by volume is approximately 21 per cent. However, when the oxygen was varied the concentration of carbon dioxide was 0.03 per cent or less. After the portions of oxygen or carbon dioxide necessary for a given percentage in a certain volume had been measured the remaining fraction of the volume was made up with nitrogen.

In the oxygen series, 22 to 24 agar cultures in Petri dishes were measured at each concentration, and 9 or 10 cultures from flasks were weighed. In the carbon-dioxide series 13 to 16 agar cultures in Petri dishes were measured at each concentration, and likewise for each concentration 3 or 4 flask cultures were weighed. It was not thought necessary to run more flask cultures in the carbon-dioxide series, as their responses were so similar to those of the agar cultures.

In these experiments where the oxygen or carbon dioxide was varied, the necessary gases were obtained from commercial pressure tanks. At the end of every 24-hour period the desired concentrations of oxygen and carbon dioxide, respectively, were made in a large graduated jar and then forced out of it into the culture jars by replacement with water. The measurements were easily made by replacing known quantities of water from the mixing jar with the gas

to be measured. The exact percentages of carbon dioxide and oxygen in the culture jars were measured with a portable gas-analysis apparatus. At the beginning of the experiments the measurements were made immediately before and immediately after a new gas supply was placed in the culture jar. It was soon found, however, that a 24-hour period of growth did not materially alter the percentage of oxygen or carbon dioxide; therefore the measurements were made only immediately after the new supplies of gases were placed in the jars. In all cases the cultures were grown at room temperature, about 21° C., for seven days.

TABLE 1.—*Growth of Ophiobolus graminis at different concentrations of oxygen*

[Age 7 days; kept at about 21° C.]

Oxygen concentration	Colonies on potato-dextrose agar		Oxygen concentration	Mycelial mats on potato-dextrose decoction		
	Number measured	Average diameter		Number weighed	Weight	
					Total	Average
Per cent		*Cm.*	*Per cent*		*Gm.*	*Gm.*
0.8	23	1.8	0.2	(a)	(a)	(a)
6.2	23	5.9	5.2	10	0.0912	0.0091
10.8	23	6.1	10.0	9	.3896	.0433
13.9	24	4.6	14.8	10	.6006	.0601
21.3	22	5.1	21.2	10	.6798	.0680

a Growth slight or none.

TABLE 2.—*Growth of Ophiobolus graminis at different concentrations of carbon dioxide*

[Age 7 days; kept at about 21° C.]

Carbon dioxide concentration	Colonies on potato-dextrose agar		Carbon-dioxide concentration	Mycelial mats on potato-dextrose decoction		
	Number measured	Average diameter		Number weighed	Weight	
					Total	Average
Per cent		*Cm.*	*Per cent*		*Gm.*	*Gm.*
0.25	14	9.04	0.90	3	0.3048	0.1016
3.59	13	8.62	3.17	4	.2630	.0658
5.19	14	4.61	5.57	3	.2696	.0899
11.80	16	6.69	11.50	4	.2276	.0569
18.75	14	5.50	18.02	4	.2120	.0530

RESULTS

GROWTH OF THE FUNGUS AT DIFFERENT CONCENTRATIONS OF OXYGEN

The results from growing cultures on potato-dextrose agar in different percentages of oxygen are given in Table 1 and are shown graphically in Figure 2. At all concentrations of oyxgen used except the lowest (0.8 per cent) the organism showed growth at the end of seven days. At 6.2 per cent and 10.8 per cent growth was slightly better than that in the controls at 21.3 per cent, whereas at 13.9 per cent the growth was slightly less than that in the controls.

In potato-dextrose decoction the fungus usually grew slightly, even at a very low oxygen concentration. The small block of agar used to inoculate the solution usually showed fuzziness even in a trace of oxygen (0.2 per cent). In general, there was a gradual diminution in growth with the decrease in oxygen. This behavior of the fungus contrasted strikingly with its action on the solid medium, where with certain exceptions, growth was approximately equal to

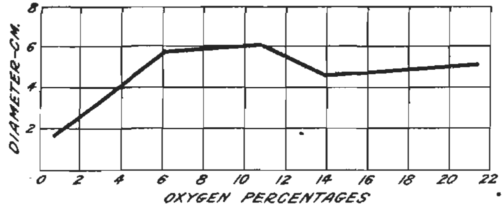

FIG. 2.—Growth of *Ophiobolus graminis* on potato-dextrose agar, at different percentages of oxygen. (Data in Table 1)

or better than the control until the oxygen was reduced below 6.2 per cent. (Table 1 and fig. 3.)

GROWTH OF THE FUNGUS AT DIFFERENT CONCENTRATIONS OF CARBON DIOXIDE

On potato-dextrose agar the fungus grew fairly well at all concentrations of carbon dioxide. In general, increased quantities of carbon dioxide usually diminished growth somewhat, but this diminution was not regular. When graphed the averages of growth measurements show a bimodal curve; that is, there was good growth

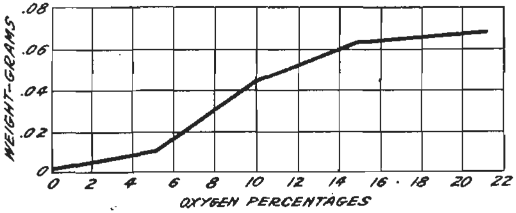

FIG. 3.—Growth of *Ophiobolus graminis* in potato-dextrose decoction, at different percentages of oxygen. (Data in Table 1)

at certain concentrations of carbon dioxide and poorer growth at an intervening concentration. The growth at 5.19 per cent was only about half as great as at 3.59 per cent. At 11.8 per cent it increased again about one-half more than at 5.19 per cent. High concentrations of carbon dioxide seemingly do not seriously impair the growth of *Ophiobolus graminis* when it is cultured on potato-dextrose agar. The growth at 16.75 per cent carbon dioxide was

about two-thirds of what it was at 0.25 per cent. (Table 2 and fig. 4.)

In general, the growth responses of the fungus in potato-dextrose decoction were similar to those on the solid medium. The curve of growth is bimodal and for the most part indicates a diminution of

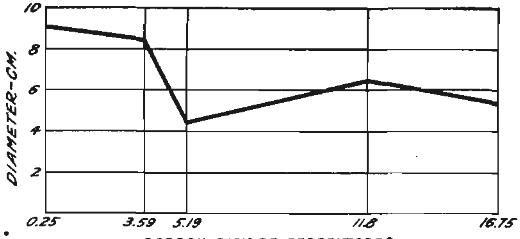

FIG. 4.—Growth of *Ophiobolus graminis* on potato-dextrose agar, at different percentages of carbon dioxide. (Data in Table 2)

growth with an increase in carbon dioxide. The two best points of growth are at 0.9 and 5.57 per cent. It may be noted that the second high point of growth has shifted toward the lower concentration as compared to that on the solid medium. The higher concentrations of carbon dioxide do not decrease the rate of growth very greatly,

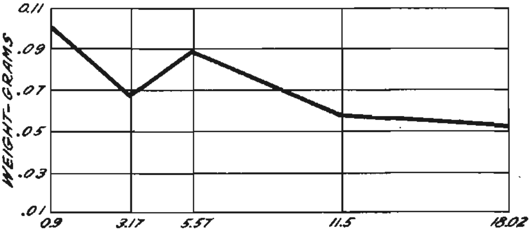

FIG. 5.—Growth of *Ophiobolus graminis* in potato-dextrose decoction, at different percentages of carbon dioxide. (Data in Table 2)

for, at the highest concentration used, growth was no less than half as great as that at the lowest concentration. One should not expect as much diminution at high percentages of carbon dioxide as at low percentages of oxygen, for oxygen is demanded in metabolism, while carbon dioxide is a by-product. (Table 2 and fig. 5.)

DISCUSSION

For a clearer understanding of the probable value of the experiments described in this paper it may be well to consider here the gaseous content of the soil. The two variable gases in the soil that mean most to the organisms that inhabit it are carbon dioxide and oxygen. There are two forms of these gases in all moist soil, namely, that dissolved in the soil water and that in the free gaseous state. It is a well-established fact that considerable quantities of carbon dioxide but only a trace of oxygen are dissolved in the soil water. This discussion is concerned with the gases found in cropped soils.

Two important pieces of work on soil gases are those of Russel and Appleyard[3] from Rothamsted, England, and of Leather[4] from Pusa, India. Russel and Appleyard worked chiefly, although not wholly, with the soil gases not in solution, while Leather included both the dissolved and the undissolved gases in his studies.

Russel and Appleyard found that the mean composition of the undissolved soil atmosphere on manured wheatland was as follows: Carbon dioxide in summer, 0.23 per cent, and in winter, 0.37 per cent; oxygen in summer, 20.74 per cent, and in winter, 20.31 per cent. On the same soil the greatest quantity of carbon dioxide ever found was 2.5 per cent in the early spring and the smallest quantity of oxygen about 18.4 per cent at the same time. In a water-logged soil the carbon dioxide was found to be as high as 9.1 per cent and the oxygen as low as 2.6 per cent. Such a condition, of course, is exceptional.

In the analyses reported by Leather both the dissolved and the undissolved gases were included. One of his representative samples was from a field growing corn (*Zea mays*). The maximum carbon dioxide content found at any time was 12.3 per cent and the minimum oxygen 6.28 per cent. The maximum quantity of carbon dioxide ever found at any time in any field used for crops was 18.4 per cent. At the same time the oxygen content was 10.6 per cent. These later analyses were made from three to nine days after a green-manure crop had been plowed under. Leather's analyses of the soil solution alone, taken when the soil was in good condition for holding oxygen, showed that, by volume, the soil solution held only 0.00607 per cent oxygen.

Ophiobolus graminis is a surface grower. If the inoculum is placed below the surface of a liquid medium the hyphae come to the surface before any extensive growth occurs. If the inoculum is placed at the surface the penetration into the liquid below is very slight. Therefore it may be assumed that *O. graminis* is more dependent for its growth and activity on the undissolved soil atmosphere than on the dissolved gases in the medium below.

From the laws of gaseous behavior, it would appear that the two soil atmospheres are not abruptly parted at the surface of water films, but that at this point there is more or less equilibrium between the two. This fact increases the difficulty of determining the percentages of the various gases to which the organism is exposed below the surface of its medium. Since *Ophiobolus graminis* is a surface grower, it is highly probable that the undissolved gases contribute

[3] RUSSEL, E. J., and APPLEYARD, A. THE ATMOSPHERE OF THE SOIL: ITS COMPOSITION AND THE CAUSES OF VARIATION. Jour. Agr. Sci. [England] 7: 1–48, illus. 1915.

[4] LEATHER, J. W. SOIL GASES. India Dept. Agr. Mem., Chem. Ser. 4: 85–134, illus. 1915.

most to its development. The small quantity of oxygen in the soil liquids, as shown by Leather's analyses, also indicates that the oxygen in the undissolved soil atmosphere is the more important. Laboratory technic has not yet been developed to the point where it is possible to determine the difference in the gas content of the surface and the deeper layers of thin aqueous films. Accordingly, one can not tell exactly what percentages of gases are in that portion of the medium in which the organism is growing.

As far as these experiments have shown, the gases ordinarily found in soils, whether free or in solution, are not present in sufficient quantity to affect the growth of *Ophiobolus graminis* very materially. Even the extreme quantities found by Russel and Appleyard on manured wheatlands in spring would not appreciably modify growth. The soil on which wheat is ordinarily grown in the wheat regions of the United States is seldom given organic fertilizers, and the carbon dioxide content therefore should rarely be as high or the oxygen as low as on the Rothamsted plots.

This paper presents but one phase of a pathological problem. To complete the study, wheat plants must be inoculated with *Ophiobolus graminis* and then grown in various concentrations of oxygen and carbon dioxide, respectively. Plans are under way to do this.

SUMMARY

Variations in the destructiveness of take-all in the field and irregularities in some greenhouse experiments suggested a series of tests to determine what effect different quantities of carbon dioxide and oxygen in the surrounding atmosphere might have on the growth of *Ophiobolus graminis*, the fungus causing take-all of wheat.

The cultures used were grown on potato-dextrose agar and potato-dextrose decoction. The two media were selected in order that the growth of the fungus might be observed on liquid and solid media when atmospheric conditions were the same.

On both the liquid and the solid media the organism grew in all the oxygen concentrations used. In the liquid medium, growth diminished gradually as the oxygen concentration decreased; on the solid medium, marked diminution did not occur until the oxygen was below 6 per cent. A very small percentage of oxygen greatly reduced growth.

The fungus grew well on both liquid and solid media when the carbon dioxide content was varied, although at the highest carbon-dioxide concentration used, 18.02 per cent, some diminution in growth occurred. In both of the carbon dioxide series; that is, the liquid-medium series and the solid-medium series, the growth curve was distinctly bimodal.

It is believed that the variations in carbon dioxide and oxygen as found in arable soils are not great enough to affect materially the growth of *Ophiobolus graminis*.

DAILY GROWTH AND OIL CONTENT OF FLAXSEEDS [1]

By A. C. Dillman

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INTRODUCTION

The yield of oil in flaxseed is of first importance to the linseed crusher because it is a large factor in the cost of oil obtained from different lots of seed. Mature flaxseed contains from 35 to 45 per cent of oil. It is important to know whether immature seed contains the same proportion of linseed oil as is found in the fully ripened seed.

Immature seed results from harvesting flax before it is fully ripened, as is sometimes necessary with late-sown flax in order to avoid possible damage from frost. It is the practice also to harvest fiber flax while the stems are still green and before the seed is fully ripe, in order to obtain a better quality of fiber. Such immature seed usually brings a lower price on the market, although there is very little definite information in regard to the oil content of immature seed. An extensive series of analyses recently completed by Coleman and Fellows (*2*)[3] indicates, however, that immature, frosted, or weather-damaged flaxseed may contain as much linseed oil (on basis of dry weight) as seed normally matured.

In order to determine at what stage of growth the oil is laid down in the developing flaxseed, the writer undertook a study of the daily growth of the seed and of the inclosing boll or capsule. This included daily measurements of the seeds from the day of flowering to full maturity, the wet and dry weights, and the oil determination of samples at regular intervals. An attempt was made, also, to determine by microchemical tests what changes occur in the early development of the seed.

The flax plant is convenient to work with in a study of this kind. The flowers normally are self-fertilized, so that uniform plants of pure lines may be obtained easily. The flowers open and pollination occurs in the early morning, and flowering of the plant continues for a period of a week or 10 days. With some varieties, especially in cool, wet weather, flowering may continue for a much longer period, so that sometimes ripe bolls and flowers may be found on the same plant. (Fig. 1.) This fact is referred to in connection with the

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[1] Received for publication July 13, 1926; issued November, 1928.

[2] The writer wishes to express his thanks to Dr. C. O. Appleman, of the University of Maryland, for assistance in outlining these experiments, and to A. C. Arny, agronomist, University Farm, St. Paul, Minn., for helpful suggestions and for the use of materials and laboratory equipment. The writer appreciates, also, the help of Frank Stevenson, who assisted in the work at University Farm, J. C. Brinsmade, jr., assistant agronomist, Office of Cereal Crops and Diseases, who obtained the material for the oil determinations at Mandan, N. Dak., and of D. A. Coleman and H. C. Fellows, Grain Division, Bureau of Agricultural Economics, for use of equipment and for assistance in making the oil determinations. The data obtained in 1926 were included in a thesis submitted to the Graduate School, University of Maryland, in partial fulfillment of the requirements for the degree of master of science, June, 1926.

[3] Reference is made by number (italic) to "Literature cited," p. 377.

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review of "Earlier investigations" herein reported. The flax boll contains 10 seeds, when all develop, and the seeds are removed easily for measurement, weighing, and analysis.

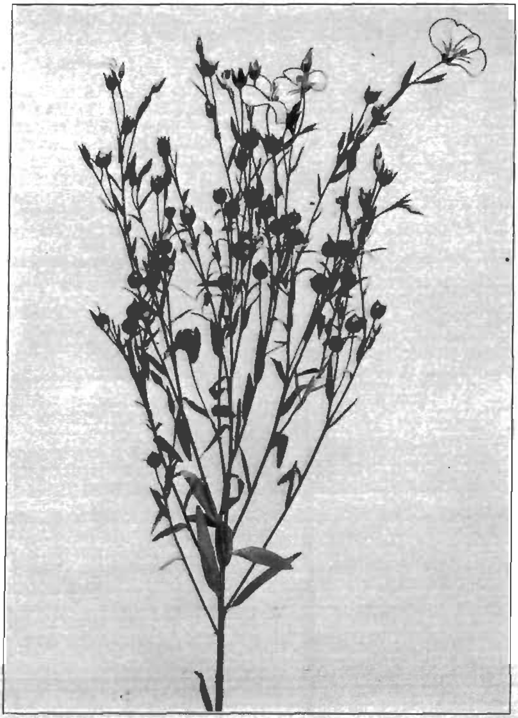

FIG. 1.—A panicle of flax, showing buds and flowers and bolls in successive stages of growth, some of the latter being fully mature

EARLIER INVESTIGATIONS

Few investigations have been made of the deposit of oil in the growing flaxseed. Most of the available studies apparently were made with all of the seed from plants harvested at different stages

of growth. As previously mentioned, the flax plant blooms for a period of one or two weeks, a few flowers appearing each day, so that bolls in successive stages of development are found on the same plant. Samples from an entire plant will contain seeds of different stages of development, depending upon the age of the individual capsules. The relative proportion of seeds of the different ages found in the samples will determine, therefore, the percentage of oil found by analyses.

Ivanow (*5*) in 1907 and 1910 determined the percentage of oil in flaxseed from plants harvested at four different stages of growth. In 1907 he obtained results ranging from 33.8 per cent of oil in samples described as "seeds green with much sap" to 41.9 per cent in "seeds fully ripe." In 1910 he obtained a much wider range in oil content. From plants harvested on July 5, about one week after flowering, the immature seeds contained 4.4 per cent of oil. From plants harvested on July 18, having "seeds unripe, sappy, green," the seeds contained 11 per cent of oil. Plants harvested on August 3, with "seeds unripe," yielded 32.5 per cent of oil, while plants harvested on August 25 with "seeds fully ripe" yielded 35 per cent of oil.

Eyre and Fisher (*3*), in a study of linseed in England in 1914, obtained a range from 21 per cent to 40.9 per cent of oil. They collected plants having green and ripe bolls and separated the seeds into four groups arranged in order of increasing degrees of ripeness and found the oil content to be as follows:

Seed quite green, 21.05 per cent.
Seed just beginning to turn brown, 30.08 per cent.
Seed wholly brown but not loose in capsule, 38.03 per cent.
Seed fully ripe, i. e., quite loose in capsule, 40.88 per cent.

Washburn (*6*), at the North Dakota Agricultural Experiment Station, found only a slight difference in the percentage of linseed oil in plants harvested August 20 when the seeds were "black to green and very light in weight" and plants harvested as late as September 26 when fully mature. The extreme range was from 38.8 to 40.8 per cent.

Coleman and Fellows (*2*) separated and analyzed immature seeds in comparison with mature sound seeds from the same bulk samples. The eight samples of immature seeds averaged 32.62 per cent of oil as compared with 41.35 per cent in the mature seeds. The unripe seeds represented only a very small proportion of the sample.

Robinson[4] working at East Lansing, Mich., in 1926, studied the oil content of seeds of a variety of fiber flax, Saginaw, during a period of 30 days, beginning on July 7 when the plants were still in bloom. The plants were pulled on successive days and dried in the open air under cover before the seeds were removed for analysis. The oil content of the seeds ranged from 22.9 per cent from plants gathered July 7 to 34.5 per cent from mature plants gathered August 5.

Bushey, Puhr, and Hume (*1*), at the South Dakota Agricultural Experiment Station, determined the oil content of flaxseed collected at five dates in 1926. The samples were taken from a plot seeded rather late, June 10. The variety is not mentioned. The oil content ranged from 29.51 per cent on August 25 when "about one-third

[4] The unpublished data from these experiments were kindly furnished the writer by B. B. Robinson, of the Office of Fiber Plants, Bureau of Plant Industry, U. S. Department of Agriculture. The experiments were carried on in cooperation with the Michigan Agricultural Experiment Station.

of the seeds were greenish in color and some flowers were still present," to 36.84 per cent on September 21 when the plants were fully mature. On October 1 when the plants were "dead ripe" the seeds contained 37.01 per cent of oil.

PRESENT INVESTIGATIONS

The investigations reported in this paper consist of two principal studies: (1) The daily growth in volume of the flaxseed and (2) the laying down of oil in the developing seed. Measurements of the daily growth of the seed were made only at University Farm, St. Paul, Minn., in 1926. The daily wet weight, dry weight, and percentage of moisture of 100 seeds were obtained at two-day and three-day intervals at St. Paul in both 1926 and 1927. The oil content of flaxseed, collected at regular intervals after flowering, was determined at St. Paul, Minn., and Mandan, N. Dak., during both years. A comparison of the rate of oil formation of seed formed during the period of early flowering (flowers tagged July 2) and seed formed during the period of late flowering (flowers tagged July 12) was made at St. Paul in 1927.

EXPERIMENTAL METHODS

Seeds of uniform and known age were obtained by tagging flax flowers on the day of blooming. This method was used by Harlan (*4*) in a study of the daily growth of the barley kernel.

The variety of flax studied was Rio (C. I. 280), a selection (L. 79) made in 1918 by H. D. Long, of the North Dakota Agricultural Experiment Station, from a sample of imported Argentine seed. This variety was chosen because it has large seeds, is uniform in type, and can be easily distinguished from other varieties, so that one can be sure of working with a uniform and pure line. The plants are short to midhigh (14 to 24 inches), with usually two or more basal branches; the flowers are large, 18 to 24 mm. wide; petals blue with darker veins; anthers blue; filaments white; style white or with a trace of light blue at the base; the bolls are large (7 to 7.5 mm. in diameter) and slow to dehisce; the false septa between the seeds are ciliate on the margin; seeds large (weight about 8 gm. per 1,000 seeds). This variety is highly resistant to wilt and immune to rust, but it is susceptible to pasmo. It often produces high yields of seed, and the seed has a high oil content. The variety, however, does not appear to endure drought or high temperature during the blossoming period, such conditions causing many flowers to blight.

Plants used for the study were growing in the border rows of plots of the chosen variety in the varietal experiments. These plots were 132 feet long and separated from adjoining plots by alleys 30 inches wide. (Fig. 2.) When the plants were from 10 to 15 inches high and in the period of early blooming, enough flowers were tagged to supply seeds for the daily measurements of growth and for oil determinations at regular intervals. The flowers were marked by tying a bit of colored woolen yarn around the flower stalk by a single loose knot, all flowers tagged on one day being marked with one color of yarn. Flowers were tagged at St. Paul in 1926 as follows:

June 18.—550 flowers, red yarn.
June 19.—600 flowers, yellow yarn.
June 22.—750 flowers, pink yarn.
June 24.—820 flowers, white yarn.

For the growth studies 15 or 18 bolls were collected each morning at 8 o'clock and taken to the laboratory as material for measurement and weight determinations. The bolls were kept in a glass jar until used, with a pad of moist cotton to prevent drying.

The lengths and diameters of 10 or more bolls were measured with a small vernier caliper, reading to 0.1 mm. The length, width, and thickness of the seeds were determined by measuring 5 seeds from each of 5 bolls, a total of 25 seeds each day. The measurements were made with a dissecting binocular fitted with a micrometer by which readings could be made directly to 0.1 mm. The 5 seeds from each boll were placed on a glass slide and the length and width

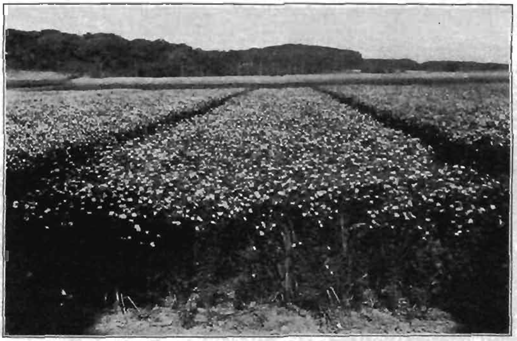

FIG. 2.—Plot of Rio flax (C. I. 280) at University Farm, St. Paul, Minn., in full bloom, June 30, 1926

recorded in succession. The seeds were then turned on edge, one at a time, with a needle, and the thickness recorded.

DAILY GROWTH IN VOLUME

The average daily growth in volume, as shown by the increased length and diameter of the boll and by the length, width, and thickness of the seed, is shown in Table 1, and graphically in Figure 3. The first measurements here recorded were made on June 18, when the boll (ovary) and ovules of 10 flowers were measured. After this date measurements of 10 bolls and of 25 seeds were made daily.

The period of growth extended from June 18, when the flowers were tagged, to July 26. The first part of the period included the season having the most hours of daily sunshine, about 15½ hours per day in the latitude of St. Paul.

The growth in volume of the boll and of the seed, as determined by the measurements, was surprisingly rapid and uniform up to the thirteenth day, when about the maximum size was attained. There appears to have been some decrease in volume from the fifteenth to the seventeenth days, inclusive, but this probably is not signifi-

cant. Apparently there was very little change in the size of the bolls or of the seeds, as determined by the dimensions, from the thirteenth day after flowering to full maturity.

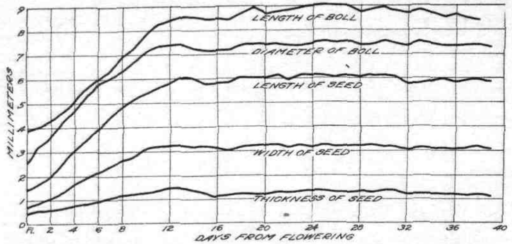

FIG. 3.—Average daily length and diameter of bolls of Rio flax (C. I. 280) and length, width, and thickness of seeds, from flowering to maturity, at University Farm, St. Paul, Minn., in 1926

TABLE 1.—*Average measurements of 10 bolls and of 25 seeds of Rio flax on successive days (except as noted) from flowering on June 18 to maturity, at University Farm, St. Paul, Minn., in 1926*

Time from flowering	Date of sampling	Measurements: Bolls, Length	Measurements: Bolls, Diameter	Measurements: Seeds, Length	Measurements: Seeds, Width	Measurements: Seeds, Thickness
		Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*
In flower	June 18	3.78	2.67	1.46	0.71	0.48
1 day	June 19	4.08	3.22	1.71	.90	.58
2 days	June 20	4.32	3.64	1.98	1.06	.60
3 days	June 21	4.70	4.31	2.48	1.36	.68
4 days	June 22	5.09	4.85	3.14	1.71	.80
5 days	June 23	5.72	5.33	3.53	1.93	.85
6 days	June 24	6.05	5.89	4.02	2.20	1.00
7 days	June 25	6.38	6.00	4.52	2.42	1.11
8 days	June 26	6.99	6.34	4.88	2.62	1.18
9 days	June 27	7.27	6.75	5.19	2.81	1.24
10 days	June 28	7.96	7.18	5.44	3.14	1.33
11 days	June 29	8.32	7.35	5.65	3.19	1.41
12 days	June 30	8.41	7.31	5.84	3.24	1.49
13 days	July 1	8.70	7.46	6.11	3.29	1.49
14 days	July 2	8.72	7.39	6.04	3.34	1.44
15 days [a]	July 7	8.44	7.14	5.80	3.23	1.23
16 days [a]	July 8	8.56	7.20	5.78	3.13	1.18
17 days [a]	July 9	8.45	7.20	5.85	3.05	1.24
18 days	July 6	8.79	7.42	6.05	3.23	1.22
19 days	July 7	8.98	7.45	6.09	3.26	1.26
20 days	July 8	8.72	7.45	6.08	3.30	1.24
21 days	July 9	8.89	7.51	6.20	3.32	1.36
22 days	July 10	8.86	7.38	6.05	3.20	1.29
23 days	July 11	8.98	7.37	6.18	3.30	1.38
24 days	July 12	9.09	7.50	6.19	3.19	1.36
25 days	July 13	9.14	7.61	6.20	3.31	1.37
26 days	July 14	9.00	7.54	6.09	3.26	1.36
27 days	July 15	9.10	7.63	6.20	3.30	1.32
28 days	July 16	8.76	7.35	6.10	3.26	1.37
29 days	July 17	8.94	7.44	6.18	3.32	1.35
30 days	July 18	8.99	7.56	6.17	3.23	1.37
31 days	July 19	8.92	7.45	6.16	3.24	1.40
32 days	July 20	8.83	7.44	5.84	3.17	1.27
33 days	July 21	8.93	7.57	5.82	3.19	1.30
34 days	July 22	8.76	7.47	5.97	3.14	1.30
35 days	July 23	8.56	7.36	6.00	3.16	1.25
36 days	July 24	8.72	7.37	5.90	3.12	1.28
37 days	July 25	8.51	7.44	6.02	3.18	1.19
38 days	July 26	8.46	7.46	6.01	3.23	1.26
Bulk ripe	Aug. 2	8.84	7.33	5.93	3.08	1.16

[a] Tagged June 22.

There was more or less variation in the size of the different bolls collected each day. This will be seen by reference to Table 2, where the actual measurements of the individual bolls and seeds are given for the first, sixth, twelfth, eighteenth, twenty-fourth, and thirtieth days of growth. As would be expected, there was an evident correlation between the size of the individual boll and of the seeds contained in it. The degree of correlation has not been determined. The data in Table 2 have been presented to indicate the range of variation encountered. Similar data were obtained for each day of the growth period, but it is considered unnecessary to present all of the detailed data in this paper.

TABLE 2.—*Measurements of individual bolls and seeds of Rio flax at definite intervals after flowering, at St. Paul, Minn., in 1926*

Sample	Measurements					Sample	Measurements				
	Bolls		Seeds				Bolls		Seeds		
	Length	Diameter	Length	Width	Thickness		Length	Diameter	Length	Width	Thickness
	June 19, 1 day after flowering						June 24, 6 days after flowering				
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*		*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
No 1	4.3	3.2	1.7	0.9	0.6	No. 1	5.6	5.5	3.9	1.9	0.9
			1.7	.8	.6				3.9	2.0	1.0
			1.6	.8	.7				3.9	2.0	1.0
			1.7	.9	.6				3.9	2.0	1.0
			1.7	.9	.5				3.9	2.0	1.0
No. 2	3.7	3.0	1.6	.7	.6	No. 2	6.0	5.8	4.1	2.2	1.0
			1.6	.8	.6				4.1	2.2	1.0
			1.6	.8	.6				4.1	2.2	1.0
			1.6	.9	.6				4.2	2.2	1.0
			1.6	.8	.6				4.1	2.2	1.0
No. 3	4.0	3.2	1.7	.9	.6	No. 3	6.1	6.0	4.3	2.3	1.0
			1.7	.8	.5				4.3	2.3	1.0
			1.7	.9	.5				4.4	2.3	1.0
			1.7	.9	.6				4.3	2.4	1.0
			1.7	.9	.6				4.3	2.4	1.0
No. 4	4.0	3.2	1.6	.9	.5	No. 4	6.2	6.0	4.2	2.2	1.0
			1.7	.9	.6				4.2	2.3	1.0
			1.7	.9	.6				4.3	2.3	1.0
			1.6	.8	.5				4.2	2.3	1.0
			1.7	.9	.6				4.3	2.2	1.0
No. 5	4.5	3.5	1.9	1.0	.6	No. 5	6.3	6.0	4.2	2.2	1.0
			1.9	1.1	.6				4.2	2.1	1.0
			1.9	1.0	.6				4.3	2.3	1.0
			2.0	1.1	.6				4.2	2.2	1.0
			1.9	1.0	.6				4.3	2.2	1.0
No. 6	4.1	3.2				No. 6	6.0	5.8			
No. 7	3.9	3.1				No. 7	6.1	5.9			
No. 8	4.4	3.3				No. 8	6.0	5.9			
No. 9	4.2	3.3				No. 9	6.1	6.0			
No. 10	3.8	3.2				No. 10	6.1	6.0			
Average	4.09	3.22	1.71	.89	.58	Average	6.05	5.89	4.16	2.20	1.00

TABLE 2.—*Measurements of individual bolls and seeds of Rio flax at definite intervals after flowering, at St. Paul, Minn., in 1926*—Continued

ample	Measurements					Sample	Measurements				
	Bolls		Seeds				Bolls		Seeds		
	Length	Diameter	Length	Width	Thickness		Length	Diameter	Length	Width	Thickness
	June 30, 12 days after flowering						July 12, 24 days after flowering				
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*		*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
			5.8	3.0	1.8				6.3	3.0	1.3
			5.7	3.1	1.5				6.3	3.2	1.2
No. 1	8.4	7.2	6.0	3.2	1.4	No. 1	9.5	7.3	6.1	3.2	1.4
			6.0	3.1	1.5				6.2	3.2	1.2
			5.8	3.4	1.5				6.2	3.2	1.2
			5.6	3.2	1.5				6.3	3.2	1.5
			5.8	3.3	1.5				6.3	3.2	1.3
No. 2	8.3	7.3	5.6	3.1	1.3	No. 2	9.0	7.3	6.1	3.1	1.4
			5.6	3.4	1.5				6.1	3.2	1.5
			6.0	3.2	1.5				6.4	3.3	1.4
			6.0	3.3	1.5				6.2	3.3	1.3
			6.0	3.3	1.5				6.2	3.3	1.4
No. 3	8.0	7.3	6.0	3.4	1.5	No. 3	9.0	7.4	6.2	3.2	1.4
			5.7	3.2	1.4				6.0	3.0	1.4
			5.9	3.2	1.6				6.0	3.3	1.3
			5.6	3.3	1.4				6.2	3.1	1.3
			5.7	3.1	1.4				6.2	3.1	1.5
No. 4	8.3	7.2	6.0	3.3	1.5	No. 4	9.3	7.1	6.1	3.1	1.3
			5.6	3.3	1.5				6.3	3.2	1.3
			5.8	3.2	1.5				6.2	3.2	1.3
			6.0	3.2	1.5				6.1	3.1	1.4
			6.0	3.3	1.5				6.2	3.3	1.4
No. 5	8.6	7.4	5.9	3.4	1.4	No. 5	8.8	7.6	6.2	3.4	1.4
			5.9	3.1	1.5				6.2	3.3	1.4
			6.0	3.3	1.5				6.2	3.3	1.3
No. 6	8.3	7.5				No. 6	9.3	7.7			
No. 7	8.7	7.4				No. 7	9.1	7.8			
No. 8	8.1	7.0				No. 8	9.0	7.6			
No. 9	8.7	7.6				No. 9	9.0	7.8			
No. 10	8.7	7.4				No. 10	8.9	7.4			
Average	8.41	7.33	5.84	3.24	1.49	Average	9.09	7.50	6.19	3.20	1.35
	July 6, 18 days after flowering						July 18, 30 days after flowering				
			6.0	3.2	1.2				5.9	3.2	1.2
			5.9	3.2	1.2				5.9	3.1	1.3
No. 1	8.6	7.3	5.9	3.3	1.2	No. 1	8.8	7.5	5.9	3.1	1.3
			5.9	3.3	1.2				5.9	3.1	1.2
			5.9	3.3	1.2				5.9	3.2	1.3
			6.0	3.3	1.3				6.2	3.3	1.5
			6.2	3.3	1.2				6.2	3.3	1.4
No. 2	9.0	7.5	6.0	3.1	1.2	No. 2	9.1	7.6	6.2	3.3	1.3
			6.2	3.0	1.4				6.2	3.3	1.4
			6.1	3.2	1.2				6.1	3.1	1.4
			6.1	3.4	1.2				6.1	3.3	1.3
			6.1	3.4	1.2				6.1	3.1	1.3
No. 3	9.1	7.5	6.1	3.3	1.2	No. 3	8.7	7.3	6.0	3.0	1.5
			6.2	3.4	1.2				6.1	3.1	1.4
			6.3	3.3	1.3				6.2	3.1	1.5
			5.9	3.0	1.2				6.3	3.3	1.4
			6.0	3.1	1.1				6.4	3.2	1.5
No. 4	8.9	7.3	6.0	3.3	1.1	No. 4	8.1	7.5	6.3	3.3	1.5
			6.0	3.2	1.2				6.3	3.2	1.4
			5.9	3.1	1.3				6.3	3.3	1.4
			6.2	3.2	1.2				6.3	3.3	1.4
			6.1	3.2	1.2				6.3	3.3	1.4
No. 5	9.0	7.3	6.1	3.2	1.2	No. 5	9.0	7.6	6.3	3.4	1.3
			6.0	3.2	1.3				6.4	3.4	1.4
			6.1	3.2	1.2				6.4	3.4	1.3
No. 6	8.9	7.5				No. 6	9.3	7.5			
No. 7	9.1	8.0				No. 7	9.0	7.6			
No. 8	8.0	7.6				No. 8	9.3	7.6			
No. 9	8.8	7.8				No. 9	9.3	7.8			
No. 10	8.8	7.6				No. 10	9.3	7.6			
Average	8.82	7.54	6.05	3.23	1.22	Average	8.99	7.56	6.17	3.23	1.37

DAILY GROWTH IN WEIGHT

The wet weight, dry weight, and percentage of moisture in flaxseed, collected each day from flowering to maturity at St. Paul in 1926, are shown in Table 3. In 1927 the samples were taken on the fifth, seventh, and ninth days after flowering and at three-day intervals thereafter to maturity. These data also are shown in Table 3. A comparison of the rate of growth, as measured by the increase in wet and dry weights, is represented graphically in Figure 4.

The curves for the daily increase in wet weight are similar to the growth curves shown in Figure 3, the wet weight reaching nearly its maximum at 12 days after flowering. After the twelfth day the

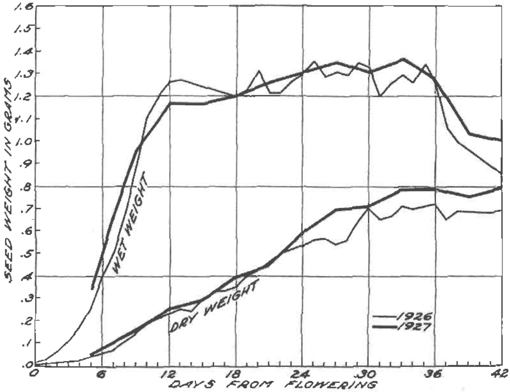

FIG. 4.—Wet and dry weights of 100 seeds of Rio flax, grown at University Farm, St. Paul, Minn., determined daily in 1926 and on the fifth, seventh, and ninth days and at three-day intervals thereafter in 1927

wet weight increased only slightly up to the thirty-sixth day, after which it declined rapidly during the period of ripening. The curves for 1926 and 1927 agree very closely.

The dry weight increased slowly during the first four days. From that time there was a more rapid and fairly uniform daily increase in dry weight up to the thirty-sixth day, when the maximum weight was attained. This point probably represents the stage of maximum growth and full maturity except for the loss of moisture in ripening. The dry weight remained constant during the process of ripening when, as noted above, the wet weight declined rapidly.

Figure 5 shows the percentage of moisture present in the seeds (based on the wet weight) from flowering to maturity. The percentage of moisture continued nearly uniform at about 85 per cent up to the eighth or ninth day. From the tenth to the thirty-fifth days there was a more or less steady decrease coincident with the increase

in dry weight. The rapid decrease in the percentage of moisture after the thirty-sixth day conforms with the decrease in wet weight during the ripening stage.

TABLE 3.—*Daily wet and dry weights of 100 seeds of Rio flax at University Farm, St. Paul, Minn., showing the percentage of moisture from day of flowering to maturity in 1926 and on the fifth, seventh, and ninth days and at three-day intervals thereafter in 1927*

Time from flowering	1926			1927		
	Wet weight	Dry weight	Moisture	Wet weight	Dry weight	Moisture
	Grams	*Gram*	*Per cent*	*Grams*	*Gram*	*Per cent*
In flower	0.0212	0.0032	85			
1 day	.0288	.0040	86			
2 days	.0600	.0098	84			
3 days	.1054	.0172	84			
4 days	.1666	.0236	86			
5 days	.2612	.0380	85	0.3378	0.0500	85
6 days	.4248	.0624	85			
7 days	.5180	.0768	85	.6735	.1042	85
8 days	.6920	.1046	85			
9 days	.8788	.1434	84	.9640	.1570	84
10 days	1.1174	.1852	83			
11 days	1.2016	.2140	82			
12 days	1.2588	.2298	82	1.1708	.2470	79
13 days	1.2732	.2500	80			
14 days	1.1112	.2440	78			
15 days	1.0820	.2928	73	1.1744	.2890	75
16 days	1.1530	.3310	71			
17 days	1.1012	.3326	70			
18 days	1.2040	.3540	71	1.2031	.3832	68
19 days	1.2252	.3930	68			
20 days	1.3076	.4200	68			
21 days	1.2206	.4646	62	1.2594	.4456	65
22 days	1.2208	.4878	60			
23 days	1.2730	.5178	59			
24 days	1.2956	.5276	59	1.3122	.5824	56
25 days	1.3604	.5576	59			
26 days	1.2876	.5600	57			
27 days	1.3174	.5428	59	1.3472	.6914	49
28 days	1.3022	.5516	58			
29 days	1.3482	.6426	52			
30 days	1.3388	.7008	48	1.3086	.7090	46
31 days	1.2056	.6514	46			
32 days	1.2680	.6764	47			
33 days	1.2942	.7102	45	1.3721	.7850	43
34 days	1.2712	.6992	45			
35 days	1.3231	.7115	46			
36 days	1.2498	.7218	42	1.2750	.7874	38
37 days	1.0768	.6532	39			
38 days	.9932	.6808	31			
39 days	.8880	.6716	24	1.1316	.7602	33
40 days	.8740	.6820	22			
42 days				1.0280	.7970	22

PERIOD OF OIL FORMATION

Perhaps the most important phase of the investigation was that concerning the time at which the oil is laid down in the developing seed. This was studied at St. Paul and at Mandan in both 1926 and 1927. In 1926 the determinations were made on samples taken at intervals of three days, beginning on the ninth day after flowering. One sample, however, was collected on the eighth day at St. Paul. In 1927, samples of seed were obtained on the fifth, seventh, and ninth days after flowering and thereafter at three-day intervals to full maturity. (Fig. 6.)

Different methods of drying the seeds before making the oil determinations were employed at St. Paul and at Mandan, but it is believed

that accurate results were obtained by both methods. At Mandan the green bolls were placed in thin gauze bags and hung in a well-ventilated attic to dry. As the weather was clear and warm, the drying was rapid, and it is probable that very little change in the oil content of the seeds occurred after the bolls were gathered. At St. Paul it was the original plan to heat the green bolls in a closed Petri dish in order to stop growth and enzyme action in the seeds, then dry the bolls in an oven, and finally remove the seeds for analysis. The heating, unfortunately, shrunk the very immature bolls so that the seeds could not be separated.

The method finally adopted was to remove the seeds from the fresh bolls. A thin section was cut from the base of the boll, exposing

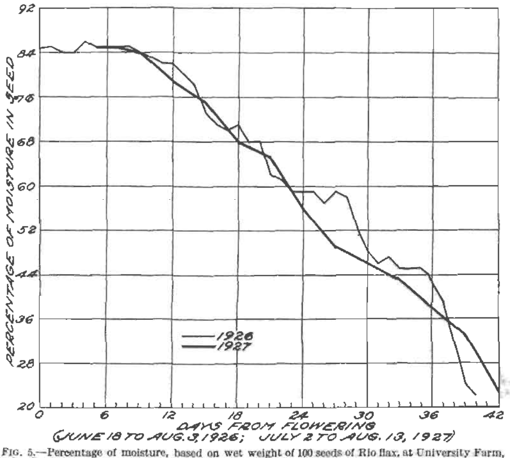

FIG. 5.—Percentage of moisture, based on wet weight of 100 seeds of Rio flax, at University Farm, St. Paul, Minn., determined daily in 1926 and on the fifth, seventh, and ninth days and at three-day intervals thereafter in 1927

the ends of the seeds. Then, by gently rolling the boll between the fingers, the seeds were pressed out. As the bolls became more woody, after 18 or 20 days of growth, it was found necessary to clip off the apex of the bolls and split down the walls to expose the seeds. The removal of the seeds from the fresh bolls in either manner was a slow process. The seeds were dried in an electric oven at 96° C.

The percentage of oil was determined by the optical method as developed by Coleman and Fellows (2). This test is based on the difference in the refractive index of linseed oil and of a solvent known as halowax oil.

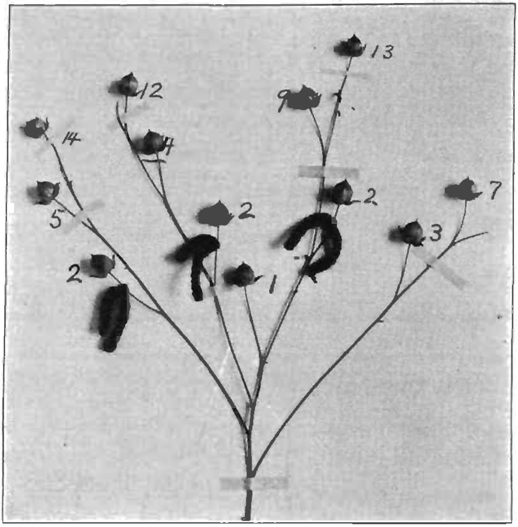

FIG. 6.—Panicle of Rio flax (C. I. 280) grown at University Farm, St. Paul, Minn., showing the dichotomous manner of branching and the actual date of blooming of all flowers (July 1-14). On July 2, 1927, more than 3,000 flowers were tagged to furnish seed of known age for the oil analyses

TABLE 4.—*Percentage of oil (on dry basis) of seeds of Rio flax at intervals after flowering to maturity at St. Paul, Minn., and at Mandan, N. Dak., in 1926 and 1927*

Time from flowering	St. Paul, 1926 [a]		St. Paul, 1927 [b]				Mandan, 1926 [c]		Mandan, 1927 [d]	
			Early bloom		Late bloom					
	Date of sampling	Oil	Date of sampling	Oil	Date of sampling	Oil	Date of sampling	Oil	Date of sampling	Oil
		P. ct.		*P. ct.*		*P. ct.*		*P. ct.*		*P. ct.*
5 days			July 7	0.83					July 19	1.40
7 days			July 9	1.10					July 21	2.39
8 days	July 1	1.84								
9 days	July 2	2.38	July 11	2.50			July 26	17.42	July 23	5.41
12 days	July 6	8.15	July 14	8.73	July 24	5.40	July 29	27.74	July 25	13.92
15 days	July 9	26.00	July 17	19.42	July 27	19.82	Aug. 1	36.10	July 28	33.48
18 days	July 12	34.66	July 20	30.98	July 30	35.03	Aug. 4	38.40	July 30	37.61
21 days	July 10	35.56	July 23	39.00	Aug. 2	40.58	Aug. 7	40.72	Aug. 3	43.23
24 days	July 13	37.50	July 26	43.75					Aug. 5	44.60
27 days	July 16	40.25	July 29	43.97			Aug. 13	42.41	Aug. 8	44.57
30 days	July 19	40.75	Aug. 1	43.34					Aug. 11	43.93
33 days	July 27	40.72	Aug. 4	42.62					Aug. 14	43.01
36 days	July 30	40.56	Aug. 7	42.88					Aug. 17	42.48
39 days	Aug. 2	40.74	Aug. 10	42.92						

[a] Flowers tagged on June 19, 22, and 24.
[b] Early bloom tagged July 2, late bloom tagged July 12.
[c] Flowers tagged July 17.
[d] Flowers tagged July 12, 13, and 14.

Two grams of each sample of seed were ground in a mortar with sand, the mortar being heated to about 70° C. to facilitate oil extraction. After the seed had been ground as fine as possible, 4 c. c. of halowax oil was added and the grinding continued for two or three minutes. The dissolved oil was then filtered through a paper filter, and the readings were made on the refractometer.

The oil content of the seeds on a dry basis is shown in Table 4 and graphically in Figure 7.

It will be seen by reference to Figure 7 that the percentage of oil in the growing flaxseed increased very rapidly for a period of about 15 to 18 days, that is, from about the seventh or ninth day after flowering to the twenty-first or twenty-seventh day. In this brief period the oil content increased from about 2 per cent to 40 per cent

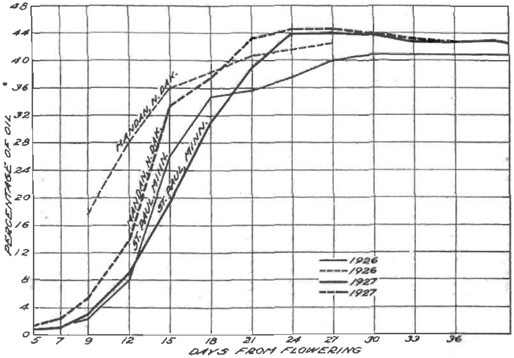

FIG. 7.—Percentage of oil (dry basis) in seeds of Rio flax (C. I. 280) at intervals from flowering to maturity at St. Paul, Minn., and at Mandan, N. Dak., in 1926 and 1927

or more in the variety studied. After nearly the maximum percentage was reached, there was slight change up to full maturity. (Fig. 8.)

There is an increase in the total oil content of the seeds, however, so long as there is an increase in dry weight. This is shown by reference to Figure 9, in which the total quantity of oil is depicted graphically as grams of oil in 1,000 seeds. The total quantity of oil is a product of the dry weight by the percentage of oil as determined at the indicated regular intervals after flowering. The graphs show that there is a rapid and very constant deposit of oil in the seeds from about the ninth day after flowering until the seeds are mature; that is, until the increase in dry weight is completed. This point was reached at about the thirty-sixth day at St. Paul, in both years, and at about the thirtieth day at Mandan, in 1927. The earlier development of the seeds at Mandan in 1926 is hereafter considered.

EFFECT OF DROUGHT AND TEMPERATURE ON SEED DEVELOPMENT

Reference to Figures 7 and 9 shows that the development of the seeds at Mandan was much more rapid in 1926 than in 1927. This was due to the extreme drought accompanied by high temperatures which prevailed at Mandan during the growing season of 1926. In both years the soil contained little moisture at seeding time. In 1926 the drought continued through the growing period, being especially severe during June and the first 18 days of July. In 1927 abundant rains occurred in May. Data on the rainfall at Mandan for each of the four months, April to July, in 1926 and 1927, and the 50-year average or normal for that locality are shown in Table 5.

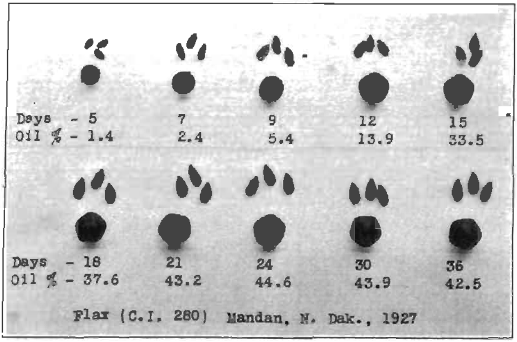

FIG. 8.—Illustration showing rapid development of bolls and seeds of Rio flax (C. I. 280) from about the seventh to the twenty-first days from flowering at Mandan, N. Dak., in 1927

TABLE 5.—*Precipitation at Mandan, N. Dak., for four months, April to July, in 1926 and 1927, compared with the 50-year average*

Month	Rainfall		
	1926	1927	Normal (50-year average)
	Inches	*Inches*	*Inches*
April	0.13	1.37	1.62
May	2.41	6.65	2.37
June	1.20	2.00	3.46
July	[a]2.19	2.37	2.29
Total	5.93	12.39	9.74

[a] The rainfall in July, 1926, nearly all occurred after July 18.

As a result of the drought in 1926, the plants were dwarfed, 10 to 14 inches high, without basal branches, and produced only three or four bolls per plant. Of more than 3,000 flowers tagged with yarn on the day of blooming less than 1,000 produced bolls. The others

failed to develop, perhaps because of nonfertilization. In the bolls that formed there were few seeds. From 305 bolls 1,158 seeds were obtained, an average of 3.8 seeds per boll, as compared with 8.9 seeds per boll in the crop of 1927.

As is often the case, the drought of 1926 at Mandan was accompanied by periods of high temperatures. Figure 10 shows graphically the maximum and mean temperatures for the periods beginning 2 days before flowering and extending to 22 days after flowering in both 1926 and 1927. High temperatures prevailed before and after the date of flowering in 1926. The average daily maximum temperature for the 24-day period covered by the graph was 84.6° F. in 1926

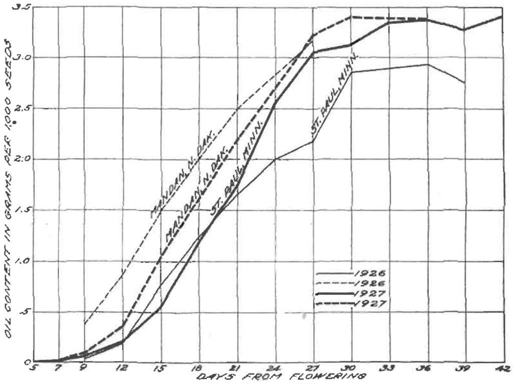

FIG. 9.—Total quantity of oil in 1,000 seeds of Rio flax at St. Paul, Minn., and at Mandan, N. Dak., at three-day intervals beginning on the ninth day in 1926, and on the fifth, seventh, and ninth days and at three-day intervals thereafter in 1927

and 79.4° in 1927, a difference of 5.2°. The average mean temperature for the period was 4° higher in 1926 than in 1927.

Drought and high temperatures, no doubt, caused the low fertility of the flax flowers. The high temperatures also probably account in part for the more rapid development of the seeds in 1926 than in 1927. Early maturity is the usual effect when drought occurs after the flowering period. Under conditions of drought the entire activity of the plant seems to function toward maturing the few bolls formed, resulting in the earlier deposit of oil as found in the 1926 crop at Mandan.

RATE OF SEED DEVELOPMENT FROM EARLY AND FROM LATE FLOWERS

In order to determine whether there was any material difference in the rate of development of seeds from the early flowers and from the late flowers on the same flax plants, flowers were tagged on July 2 (early flowers) and on July 12 (late flowers) at St. Paul in

1927. Bolls were collected at 3-day intervals during the period of most rapid oil formation as previously determined—namely, at 12, 15, 18, and 21 days after flowering—and the dry weight, the percentage of oil, and the total quantity of oil were determined. These data are given in Table 6.

Reference to Table 6 shows no consistent difference between the rate of development of the seeds from the early flowers and those from the late flowers as determined by the weight of 100 seeds. There were some variations in the percentage of oil in seeds of the same age and in the total quantity of oil, but these variations were not consistent and probably not significant. The entire growing season was favorable, however, and the plants were not checked in

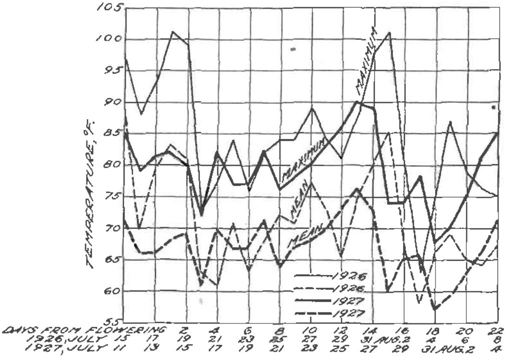

FIG. 10.—Maximum and mean daily temperature at Mandan, N. Dak., during periods of 24 days beginning 2 days before the flowering of flax and extending to 22 days after flowering, in 1926 and 1927

growth at any time, as is proved by the high yield of 26.3 bushels per acre obtained from the plots of this variety, so it is probable that no significant difference should be expected.

TABLE 6.—*Dry weight of 100 seeds, percentage of oil (dry basis), and total quantity of oil in 1,000 flax seeds obtained at St. Paul, Minn., from flowers tagged in the early-bloom period (July 2) and in the late-bloom period (July 12) on the same plants in 1927*

Time after flowering	Dry weight of 100 seeds		Percentage of oil in seeds		Total quantity of oil in 1,000 seeds	
	Early bloom	Late bloom	Early bloom	Late bloom	Early bloom	Late bloom
	Gram	*Gram*	*Per cent*	*Per cent*	*Grams*	*Grams*
12 days	0.2470	0.1977	8.73	5.40	0.216	0.106
15 days	.2890	.2900	19.42	19.82	.561	.575
18 days	.3832	.4026	30.98	35.03	1.187	1.410
21 days	.4456	.4550	[a] 39.00	40.58	1.738	1.846

[a] Interpolated from other determinations. (See fig. 7.)

It is possible, however, that a difference in the rate of oil deposition between the early-formed seeds and the late seeds would occur if there were a marked difference in temperature during the two periods of seed development. A period of higher temperatures for 10 to 20 days after the blossoming period would hasten the development of the late-formed bolls and seeds as compared with the early ones if the latter made their growth during a cool cloudy period. This fact was suggested by small differences observed in the rate of growth of bolls and seeds of the same age (time from flowering) but collected several days apart at St. Paul in 1926.

COMPARATIVE DEVELOPMENT OF SEEDS FROM ALL FLOWERS AND FROM EARLY FLOWERS

As mentioned under "Earlier investigations," the studies heretofore made on the oil content of immature flaxseed have been based on samples obtained by harvesting (pulling) and drying the plants and threshing out all the seeds for oil analysis. Such bulk samples are a mixture of seeds of various ages, depending upon the age of the individual bolls in days after flowering. A thousand seeds 5 days old have about the same mass (dry weight) as 100 seeds 24 days old. In order to compare the development of the bulk seeds with the seeds of known age in days, from the same or similar plants, typical plants were pulled at three-day intervals and dried in the laboratory. Later the yield of seed from 50 plants, the weight of 100 seeds representing a uniform sample, and the percentage of oil were determined. Data obtained at both St. Paul and Mandan in 1927 are presented in Table 7.

TABLE 7.—*Yield of seed per plant, weight of 100 seeds, and percentage of oil from flax plants harvested at three-day intervals from date of last blooming to maturity at St. Paul, Minn., and at Mandan, N. Dak., in 1927*

Place and date of harvest [a]	Stage of growth	Weight of—		Percentage of oil (dry basis)
		Seed per plant	100 seeds	
St. Paul, Minn.:		*Grams*	*Gram*	
July 14	Date of last bloom; dry seeds very thin, green or colorless	0.06	0.142	23.54
July 17	Dry seeds still green or colorless, a few brown	.14	.227	29.68
July 20	Plants still green. Half of dry seeds brown, but very thin	.19	.320	33.15
July 23	Plants green; over half of dry seeds brown, others green or colorless.	.20	.361	35.80
July 26	Plants green; a few ripe bolls; seeds brown, a few colorless	.26	.547	39.61
July 29	Plants green; many ripe bolls; seeds brown, a very few thin	.38	.589	40.52
August 1	Plants still green; most bolls ripe; seeds brown, some thin	.38	.601	41.88
August 7	Plants partly brown, ripening; bolls ripe; seeds brown	.36	.740	40.09
August 10	Plants nearly ripe; seeds brown, plump	.45	.753	40.31
August 13	Plants fully ripe	.45	.772	40.03
Mandan, N. Dak:				
August 3	Date of last bloom; dry seeds very thin, green or colorless	.35	.291	33.85
August 6	Plants green; about half of seeds brown, others colorless	.53	.336	34.91
August 9	Plants green; nearly all seeds brown, but thin, a few colorless.	.53	.446	36.88
August 12	Plants green; most seeds brown, a few colorless	.80	.489	38.72
August 15	Plants ripening; many bolls brown; seeds all brown	.97	.640	41.30
August 18	Plants nearly ripe; most bolls ripe; seeds brown, a few thin and immature.	1.07	.660	41.47

[a] The first flowers appeared at St. Paul about June 28, first full bloom July 2, last bloom July 14; first flowers at Mandan about July 8, full bloom July 14, last bloom August 3.

The plants at St. Paul were grown in drilled plots (rows 7 inches apart) and were pulled from the second row from the border of the plot. The plants at Mandan were grown in cultivated rows 12 inches apart. This difference in culture accounts for the greater yield of seed per plant of the plants grown at Mandan.

At St. Paul the weight of 100 seeds increased from 0.142 gram on July 14, the date of last blooming, to 0.772 gram on August 13, when the plants were ripe. The proportion of oil increased from 23.54 per cent to 40.03 per cent during the same period. A similar development is shown in the samples from Mandan.

The seeds of uniform age, obtained by tagging flowers on the day of blooming, show a more rapid increase in oil content than the bulk seeds from all flowers. This is shown graphically in Figure 11.

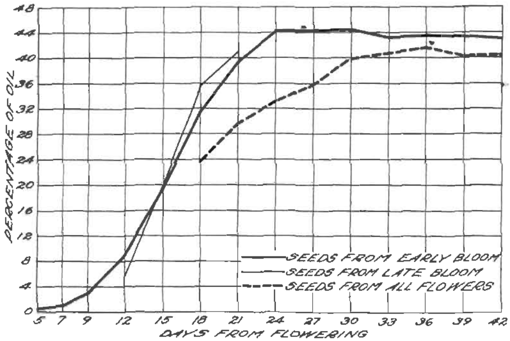

FIG. 11.—Percentage of oil in flaxseed from the early full bloom (flowers tagged July 2), from the late bloom (flowers tagged July 12), and from bulk seeds (all flowers). The first bulk sample was obtained at St. Paul, Minn., in 1927 by pulling the plants on July 14, about 18 days after the first flowers appeared

MICROCHEMICAL OBSERVATIONS OF PERIOD OF OIL FORMATION

Observations were made at St. Paul, by means of microchemical tests, of the changes that occur in the young seeds. The yellow stain, Sudan III, is specific for oil and was used in staining sections of seeds daily for several days after flowering.

In seeds three and four days after flowering, the tissues of the cotyledons contained many clear white grains or crystals, which stained black with iodine, indicating that they were starch grains. The integuments of the seed, the seed coat, also contained starch at this stage. No positive test for oil was obtained.

In seeds six days after flowering green chlorophyll was present in the cotyledons. The cotyledons stained distinctly with Sudan III, but stained only slightly with iodine. This would indicate that the starch which was present in seeds four days after flowering had been converted into oil. No tests for oil were obtained before chlorophyll

appeared. A test for oil always was obtained where chlorophyll was present, the seeds staining distinctly with Sudan III.

These tests were merely preliminary, and it is planned to investigate further this phase of the subject.

The brown coloring matter of the seed coat, which appears in a single cell layer of the integuments, did not appear in the freshly gathered seeds until the seeds were nearly mature, that is, from the twenty-seventh or thirtieth day after flowering at St. Paul in 1926. The color apparently is present earlier and develops in seeds 12 to 18 days old as the wet seeds dry out, but it is not visible in the fresh seeds. The brown pigment is some form of tannin probably in combination with a resinous or fatty substance, presumably linseed oil, according to an unpublished report by Albert Mann, formerly of the Bureau of Plant Industry, United States Department of Agriculture.

GERMINATION OF IMMATURE FLAXSEED

The samples of seed collected at Mandan in 1927 were tested for germination. These seeds were obtained from bolls collected at three-day intervals after flowering and dried in a well-ventilated attic room. The germination test was made on moist blotters in a moist chamber kept at 20° C. at night and at 30° during the day.

At the end of 6 days no germination had occurred in seeds harvested at 9 or 12 days after flowering. There was 38 per cent germination in seeds harvested at 15 days, 80 per cent at 18 days, 90 per cent at 24 days, and an average of 95 per cent in four samples harvested at 27 to 36 days after flowering. A photograph of the germinated seeds is reproduced in Figure 12.

There was no germination of seeds harvested before 15 days after flowering. Seeds after 15 days' development, had attained about one-third of their mature dry weight and contained 33.48 per cent of oil. The fresh seeds, at 15 days, had reached their maximum growth in volume, the cotyledons were green with cholorophyll, and the embryo was formed completely.

DISCUSSION AND CONCLUSIONS

This paper presents data showing the daily growth of flaxseed of one variety, Rio (C. I. 280), from flowering to maturity at University Farm, St. Paul, Minn., in 1926, and of the time of oil formation in the developing seed as determined at St. Paul in 1926 and 1927 and at Mandan, N. Dak., during the same years. This is a subject of some interest as a study of plant metabolism and of practical importance in its bearing on the question of how early the flax crop can be harvested without loss in yield or quality of seed.

The data show that the growth of flaxseed, as determined by measurements of length, width, and thickness, is comparatively rapid. The seed increased proportionately in all three dimensions during the same period, reaching a maximum volume at 12 to 14 days after flowering. The volume remained more or less constant until the thirty-first day, after which a slight decrease occurred during the ripening stage. The growth of the flaxseed is different, therefore, from that of the barley kernel as determined by Harlan (*4*). In the barley kernel the growth in length is completed by the seventh day

after flowering, the lateral diameter continues to increase to about the seventeenth day, and the dorsiventral diameter increases until the grain is nearly mature at 24 days after flowering.

The wet weight of 100 seeds, as would be expected, increased with the increase in volume and reached nearly its maximum at 13 days. (Fig. 4.) It decreased rapidly after the thirty-fifth day, during the ripening process.

The growth of the seed, as determined by the daily increase in dry weight of 100 seeds, continued uniformly for a period of 33 days.

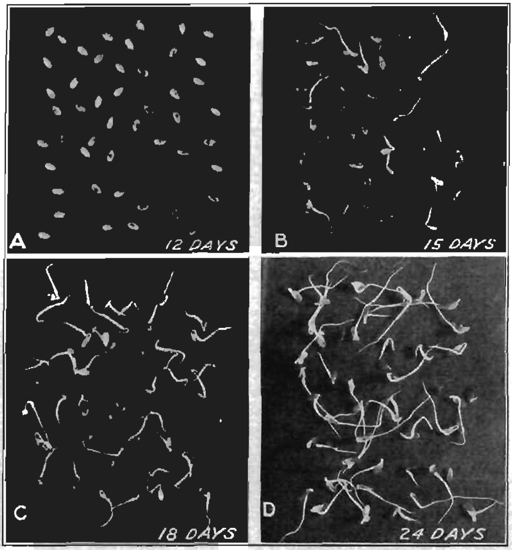

FIG. 12.—Germination of flaxseed harvested at Mandan, N. Dak., in 1927: A, 12; B, 15; C, 18; and D, 24 days after flowering

Thereafter the weight remained constant to the end of the ripening period at 39 or 40 days. This would indicate that there would not be any loss in total yield of seed if the crop were harvested somewhat "on the green side," that is, before the bolls and stems were fully brown.

The most rapid formation of oil, based on the percentage of oil in the dry seeds, begins at about the seventh day after flowering and continues for a period of 15 to 18 days. After the maximum percentage is reached there is little or no significant change up to full

The total oil content of the seeds continues to increase with the increase in dry weight. The maximum oil content of the seeds is coincident, therefore, with the maximum dry weight. This point apparently is reached some six to nine days before the seeds are fully ripe and dry enough to harvest as usually practiced.

Where fiber flax is grown it is usually harvested somewhat green. Where flax is grown for seed, the straw often is used for the manufacture of coarse tow and sometimes as feed for livestock. For both of these purposes it is more valuable if harvested while the stems are still somewhat green. The data indicate that flax may be harvested before the plant is ripe and dry without sacrifice in weight of seed (that is, in yield per acre) or in percentage yield of oil.

Severe drought at Mandan in 1926 dwarfed the vegetative growth of the flax plants, caused fully two-thirds of the flowers to blight without setting bolls, and reduced the number of seeds per boll. The drought apparently hastened the time of oil deposition and of maturity as compared with the normal moisture conditions which prevailed in 1927. This may have been due partly, also, to the somewhat higher temperatures that prevailed at Mandan in 1926. The actual time when growth ceases must depend on the climatic conditions of temperature, humidity, and especially of soil moisture.

The brown color of the seed coat does not appear in the freshly gathered seed until the seed is nearly mature, that is, at approximately the time when the maximum dry weight is attained. This was at about the thirty-sixth day after flowering at St. Paul in 1926. The coloring substance, some form of tannin, evidently is present in the seed coat at an earlier stage, because it appears on drying the seeds collected at 12 to 18 days after flowering.

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